STUDIES ON THE ECTENDOTROPHIC MYCORRHIZA OF PINE

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Acknowledgments

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Introduction

Review of Literature

From the early days of mycorrhizal research two types of mycorrhiza have been distinguished, ectotrophic and endotrophic. The ectotrophic mycorrhiza occurs on many of the commonest forest trees of the temperate zones. Its characteristic feature is that the fungal hyphae do not penetrate the root cells but grow as a dense mantle on the surface of the short roots and as a network (Hartig net) between the cortical cells. MELIN (1917) pointed out that in an ectotrophic mycorrhiza some hyphae may also be found inside the cortical cells and called a type having structural features of both the ectotrophic and endotrophic mycorrhiza, ectendotrophic (MELIN 1923a, p. 108). In fact, according to the literature review by MELIN (1923a) descriptions of the ectendotrophic mycorrhiza had already earlier been published. Thus, MÜLLER (1903) wrote that the spruce mycorrhizae are ectotrophic while the pine has both ectotrophic and endotrophic mycorrhizae, the latter probably corresponding to those called ectendotrophic by MELIN. Similar mycorrhizae had been described by v. TUBEUF (1903) in *Pinus cembra*, by PEKLO (1913) in pine and spruce, and by MCDOUGALL (1914) in basswood (*Tilia americana*). In addition to pine, MELIN (1922 and 1923 b) found ectendotrophic mycorrhizae on larch and birch. Later on, RAYNER (1927) stated that the tuberous mycorrhiza of *Arbutus unedo* is ectendotrophic. Furthermore, the occurrence of ectendotrophic mycorrhizae in *Pirolaceae* has been reported (SCHAEDE 1948).

MELIN (1917) also introduced the term pseudomycorrhiza to designate a short root in which fungal hyphae grow intracellularly in the cortex but the characteristic structural features of the ectotrophic mycorrhiza (mantle, Hartig net, and hypertrophy of the cortical cells) are lacking. The pseudomycorrhiza differs from the endotrophic mycorrhiza in that the association is not symbiotic but the fungus is a weak parasite and no digestion of hyphae takes place in the host cells.

In the more recent literature, reports on ectendotrophic mycorrhizae are sporadic and often contradictory. In textbooks and popular articles the descriptions of ectendotrophic mycorrhiza are based mainly on the early papers of MELIN. Many recent publications recognize only the ectotrophic and endotrophic mycorrhiza, without mentioning the intermediate type.

Endrigkeit (1937) and Levisohn (1963) in Germany and England did not find ectendotrophic mycorrhizae such as have been described by Melin. On the other hand, Rayner (1934) reported ectotrophic mycorrhizae as almost invariably having intracellular hyphae too. Bergemann (1955) observed intracellular infection to be very common on pine, spruce, and larch seedlings, especially in poor soil. According to Björkman (1940, 1942), the ectendotrophic mycorrhiza is common, especially on pines on poor sites; on spruce it is not so typically developed and occurs less frequently. Harley (1959) has found ectendotrophic mycorrhizae on beech.

In the American literature the ectendotrophic mycorrhiza is seldom mentioned. McDougall and Jacobs (1927) reported it on lodgepole pine in the Rocky Mountains, and McComb (1943) found it on pine seedlings in nurseries in Iowa. Hacskaylo and Palmer (1957) considered it uncommon, while Goss (1960) found it very common on ponderosa pine in Nebraska, both on young seedlings and on mature trees. It is also known on pine in Australian nurseries (Young 1938).

The main reason for the contradictory information about ectendotrophic mycorrhizae is the fact that different workers have adopted different meanings for the terms ectotrophic and ectendotrophic mycorrhiza and pseudomycorrhiza. Pseudomycorrhiza, in particular, is a vague term which should be used with reservation. Thus, for instance, Levisohn (1963) requires that the ectotrophic mycorrhiza should have a mantle and classifies all short roots with a Hartig net and without a mantle as pseudomycorrhizae. In the papers of Björkman (1942, p. 22), Bergemann (1955, pp. 194—197), and Goss (1960, p. 13) there are figures of short roots with inter- and intracellular infection and without a mantle, which the authors call ectendotrophic mycorrhizae, while Rayner (1934, Pl. XII, Fig. 4) and Levisohn (1963, Taf. I) present photographs of similar structures, calling them pseudomycorrhizae. Melin (1923a, p. 84) stated that both ectotrophic and ectendotrophic mycorrhizae may be devoid of a mantle. The mantle of ectendotrophic mycorrhizae is usually described as thin or lacking; Melin (1923a, p. 105), however, claimed that even the tuberous pine mycorrhiza (»Knollenmykorrhiza»), with its thick mantle, was ectendotrophic. Considering the common occurrence of intracellular infection and the difficulty of detecting it, it is doubtful whether ectotrophic mycorrhizae *sensu stricto* exist at all (Melin 1923a, p. 107).

Departing from the usual terminology, Lobanow (1960) has called all mycorrhizae with a mantle and Hartig net ectendotrophic, irrespective of the presence or absence of intracellular infection. Correspondingly, in the ectotrophic mycorrhiza the fungus grows only outside the root as a mantle. Thus, the ectotrophic mycorrhiza of Lobanow corresponds approximately to the peritrophic mycorrhiza of Jahn (1934).

According to the original description given by Melin (1917, pp. 358—360),

in pseudomycorrhizae the fungal infection is intracellular only. Both a mantle and Hartig net are absent, and there is no hypertrophy of the cortical cells. Later on, however, Melin (1927, p. 461) remarked that an intercellular network may also be present in pseudomycorrhizae. As was stated above, Rayner (1954) and Levisohn (1963) included in the pseudomycorrhizae short root with a distinct Hartig net, while a mantle and sometimes even intracellular infection might be missing.

Slender, dark-colored and unbranched short roots, without a visible mantle or outer mycelia, are perhaps usually included in pseudomycorrhizae when the classification is based on macroscopic examination. Microscopic examination of such short roots, however, may reveal a Hartig net and even a thin mantle, although the cortical cells are not hypertrophic (Mikola & Laiho 1962). Furthermore, »pseudomycorrhizae» may include old mycorrhizae where the cortex is dark and collapsed and may even have disappeared.

There are also divergent opinions on the commonness of the pseudomycorrhiza. Several authors have claimed all short root of conifers to be infected by fungi, i.e. either mycorrhizae or pseudomycorrhizae (Melin 1927; Björkman 1942) while Goss (1960) found relatively few mycorrhizae and no pseudomycorrhizae at all, i.e. the majority of the short roots were uninfected.

The essential difference between mycorrhiza and pseudomycorrhiza should be physiological, i.e. in mycorrhizae the relationship of the host and the fungus is symbiotic, while in pseudomycorrhizae the fungus is a parasite and the host is not benefited by the association. According to Rayner (1934), there are no morphological or anatomical differences between the ectendotrophic mycorrhiza and the pseudomycorrhiza; the decisive difference is that in ectendotrophic mycorrhizae the intracellular hyphae disintegrate and are digested by the host cells, while no digestion of hyphae takes place in pseudomycorrhizae. »Indeed, one of the most difficult problems that faces the modern student of tree mycorrhiza is to define with precision the boundary between normal ectendotrophic structure and intracellular infection of a pseudomycorrhizal kind due to upset of the balanced normal relation» (Rayner 1934, p. 101).

By microscopic examination without physiological experiments, however, it is not possible to decide with certainty whether the association is beneficial, harmful, or insignificant for the tree. A classification that can be used in microscopic study must be based on such anatomical features as can be distinguished without possibility of dispute. The presence or absence of a Hartig net is such a feature. Therefore, in this paper all short roots with a Hartig net are called mycorrhizae. If, in addition, intracellular hyphae are present, the mycorrhiza is called ectendotrophic, otherwise ectotrophic. A mantle may be present or absent in both types.

The term pseudomycorrhiza is confusing. In this category different workers may have included short roots with a Hartig net (i.e. mycorrhizae in the sense

of the present paper), intracellularly infected short roots, old, dead, or poorly developed mycorrhizae, and perhaps also uninfected short roots (absence of root hairs is no reliable criterion of fungal infection). Maybe the term »pseudomycorrhiza» should be abandoned, as LEVISOHN (1963) has suggested. Instead, in contrast to mycorrhizae, non-mycorrhizal short roots can be used, this category including both uninfected and solely intracellularly infected short roots.

The structure of the ectendotrophic mycorrhiza, being intermediate between that of the ectotrophic mycorrhiza and pseudomycorrhiza on the one hand and of ectotrophic and endotrophic mycorrhiza on the other, has provoked two kinds of theories as to its physiological role. First, if the ectendotrophic mycorrhiza is considered as something between the ectotrophic mycorrhiza and the pseudomycorrhiza, then probably the symbiotic balance has been disturbed, the fungus behaves as a parasite and the association may be more harmful than beneficial for the tree. Such an explanation is supported by the fact that the ectendotrophic mycorrhiza (i.e. strong intracellular infection and the absence of a mantle) is most commonly seen on stunted seedlings in poor soils (BJÖRKMAN 1942; BERGEMANN 1955). On the other hand, the assumption has been made that somewhat the same kind of nutritional relationship could prevail in both the ectendotrophic and endotrophic mycorrhiza, i.e. fungal hyphae are digested inside the host cells; in other words, the ectendotrophic mycorrhiza would be both morphologically and physiologically an intermediate type between ectotrophic and endotrophic mycorrhizae.

One of the central problems of mycorrhizal research today is whether different species of mycorrhizal fungi differ from each other physiologically and whether some species are more beneficial for the tree than others. The solution of this problem would make it possible, in silvicultural and nursery practice, to promote the most useful species by inoculation or other means. In this respect the ectendotrophic mycorrhiza and fungi concerned are of particular importance.

So far it is not known whether specific ectendotrophic mycorrhizal fungi exist or whether the same species are able to form both ectotrophic and ectendotrophic mycorrhizae, depending on environmental conditions.

Ectendotrophic Mycorrhiza in some Finnish Nurseries

The ectendotrophic mycorrhiza and its characteristic features first attracted attention when the effects of some biocides on mycorrhizal development in forest nurseries were studied (LAIHO & MIKOLA 1964). Then the fact was discovered that pine mycorrhizae were invariably ectendotrophic in one of two neighboring nurseries (Hyytiälä old nursery) and ectotrophic in the other

(Hyytiälä new nursery). At the same time the observation was made that spruce mycorrhizae were ectotrophic in both nurseries.[1]

The nutrient level of the two nurseries was rather low, i.e. considerably lower than in Finnish nurseries on the average, but no essential differences in soil properties between the two nurseries were observed (Table 1). Therefore, the most probable explanation for the difference seemed to be in the species of fungi, i.e. that some fungus forming ectendotrophic mycorrhizae predominated in the old nursery and was absent in the new one. This hypothesis was supported by the fact that the new nursery was on an old field which for decades had been in farm use and at the beginning of the experiment (1960) was under coniferous seedlings for the first year, while in the old nursery conifers had been grown for seven years in succession.

Table 1. Soil properties in Hyytiälä nurseries

	Loss on ignition %	pH	N %	Exchangeable			
				K_2O	CaO	MgO	P_2O_5
				mg/100 g			
Old nursery	5.2	5.5	0.18	5.7	85	2.5	1.3
New nursery ..	6.7	5.7	0.22	11.5	114	8.5	1.2

Similarly, pine mycorrhizae were ectendotrophic and spruce mycorrhizae ectotrophic in Punkaharju nursery, belonging to the Finnish Forest Research Institute, where experiments with fungicides and herbicides were conducted at the same time and where conifers had been grown for decades.

These early observations both prompted and provided a good opportunity for further investigations into the ectendotrophic mycorrhiza and the fungi involved. This study is aimed mainly at finding out whether the difference of ectotrophic and ectendotrophic mycorrhiza depends on fungal symbionts or environmental conditions. Furthermore, the occurrence of the ectendotrophic mycorrhiza in Finland under various conditions was studied and experiments on the physiology and ecology of the mycorrhiza and the fungal partner were conducted.

Structure of the Ectendotrophic Mycorrhiza

The appearance and morphology of the ectendotrophic mycorrhiza was very much the same in all instances where it was found — in Hyytiälä old nursery and other nurseries and in the pot cultures of greenhouse experiments (Figs. 1—5). The color is light brown and darkens as the mycorrhizae grow older. The surface is smooth, and no mantle or outside mycelia can be seen with naked

[1] Neither were typically ectendotrophic spruce mycorrhizae found in other nurseries or in samples taken from natural stands. Intracellular hyphae can be found in spruce mycorrhizae, it is true; they grow, however, only in degenerating cortical cells of old mycorrhizae when the symbiotic stage of the association has probably been passed. Therefore further study was concentrated on pine.

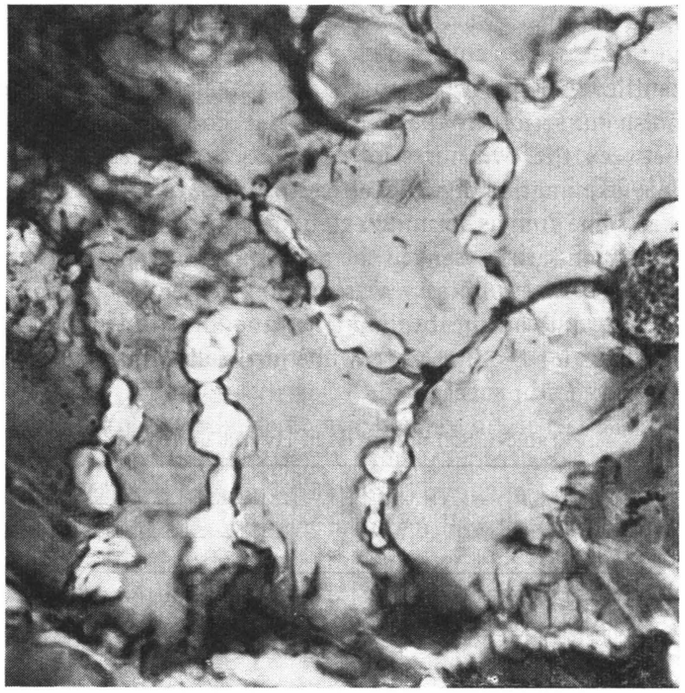


Fig. 1. Tangential section of the surface layer of an ectendotrophic mycorrhiza of pine. The fungus only grows intercellularly between the surface cells.[1] From a greenhouse experiment in sterilized soil. The pot was inoculated on July 27, 1963, and seedlings were harvested on Oct. 4. × 650.

eye. The mycorrhizae are usually dichotomous with two or more branches, often even coralloid clusters; the ectendotrophic structure, however, is usually present in young roots before branching. The ectendotrophic mycorrhizae are rather slender, only slightly thicker than the non-mycorrhizal short roots (Table 2).

Table 2. The average thickness of ectendotrophic mycorrhizae and non-mycorrhizal short roots, and their stelar and cortical parts in greenhouse experiments of 1963 (pp. 34—48).

	Thickness of			Number of determinations
	short root, mm	cortex μ	stele μ	
Ectendotrophic mycorrhizae				
dichotomous	0.38	135	110	98
unbranched	0.36	118	121	36
Non-mycorrhizal short roots	0.32	104	107	87

[1] The staining procedure was as follows: Roots were fixed and preserved in Navashin's solution. After mounting in paraffin, sections 7 μ thick were cut. They were then stained with safranine (0.5 ml of 1 % solution in 100 ml of water) overnight and counterstained with fast green (saturated solution in a mixture of methyl cellulose, absolute alcohol, and terpineol) for 5—20 min.

The fungal mantle is very thin (4—10 μ) or completely lacking. In the cortex two layers can be distinguished. In the outer layer the cells are flattened, filled with tannin, and the fungus grows mainly intercellularly; the coarse Hartig net appears distinctly in tangential sections near the root surface (Fig. 1). In the deeper layer of the cortex the cells are hypertrophied and the fungus grows there both inter- and intracellularly (Figs. 2—3). The hyphae of the Hartig net are coarse (4—10 μ thick) and bulbous. The intracellular hyphae are up to 15 μ thick, with short cells, winding, branching, and sometimes almost filling the cortical cells.

The first sign of a fungal infection in young short roots is the Hartig net between the cells near the surface. As the short root grows, the intercellular net follows behind the meristem, and intracellular hyphae appear 2—3 cells further back. Particularly heavy intracellular infection is often visible in the base of the short roots or in the region where infection had first started (Fig. 4), as well as between the dichotomous branches.

Intracellular hyphae do not injure the cortical cells; both host cells and intracellular hyphae were observed to live at least one year after the commencement of infection; even the nuclei of such heavily colonized cortical cells were clearly visible in stained sections (Fig. 5). The question of the digestion of intracellular hyphae is somewhat obscure. In some sections these hyphae looked as if they were melting into an amorphous mass; on the other hand, in many 2-year-old mycorrhizae the intracellular hyphae were as clear and distinct in the old as in the young portions.

As a mycorrhiza ages, both cortical cells and intracellular hyphae in its oldest part die and disappear simultaneously. Comparison of ectotrophic and ectendotrophic mycorrhizae proved that the life time of the mycorrhizal cortex is approximately the same in both types. The cortex with fungal net and mantle usually collapses after functioning for two growing seasons, while the cortex of non-mycorrhizal short roots lasts a much shorter time.

The above ectendotrophic mycorrhiza is identical with those described by BJÖRKMAN and Goss, at least as far as can be concluded from microphotographs (BJÖRKMAN 1942, Fig. 5; Goss 1960, Fig. 7). In England, RAYNER (1934) described two types of ectendotrophic pine mycorrhizae, the latter of which corresponds to the above type. (»Into the other class fall the by no means infrequent cases in which the cells of the cortex are invaded and often filled by haustorial-like hyphae that have forced their way into the cells from the network of mycelium that envelops them. In section, this condition may affect a few cells only or practically all of the cortical tissue.»)

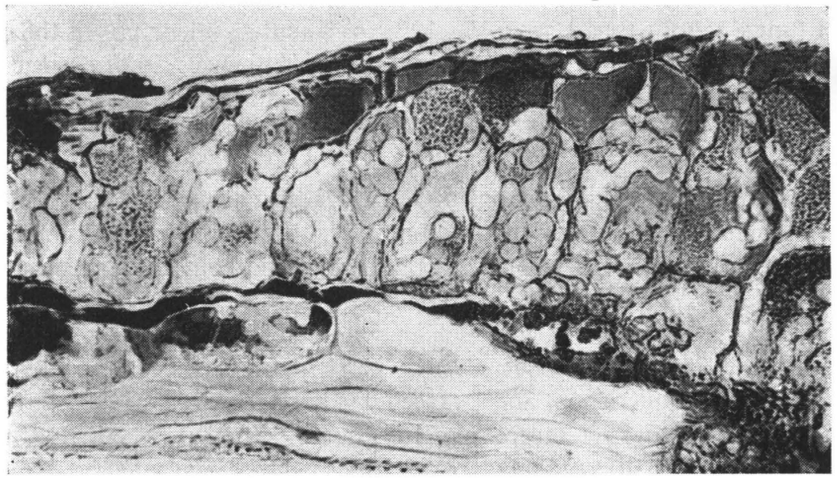


Fig. 2. Longitudinal section of an ectendotrophic pine mycorrhiza. (From a greenhouse experiment of 1963.) × 380.

Fig. 3. Longitudinal section of an ectendotrophic pine mycorrhiza. (Hyytiälä old nursery, 1961.)

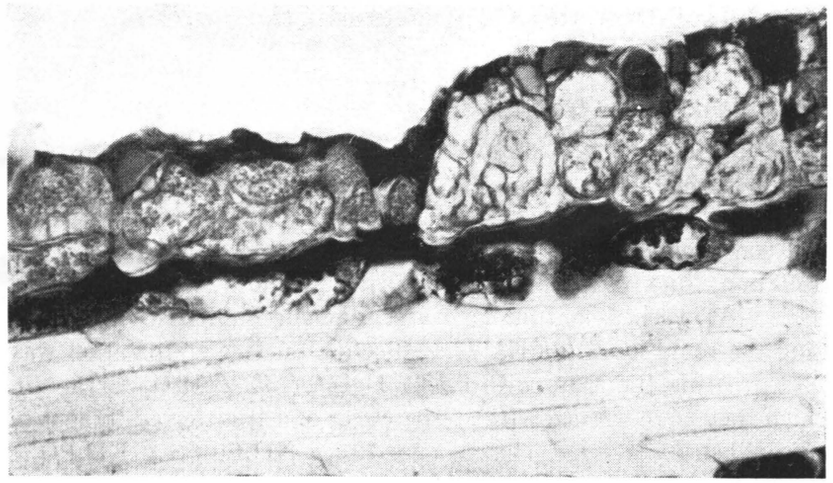


Fig. 4. An early stage of ectendotrophic infection (from a greenhouse experiment of 1963). The seedling was inoculated with pure culture on July 27, and removed on August 27. × 350.

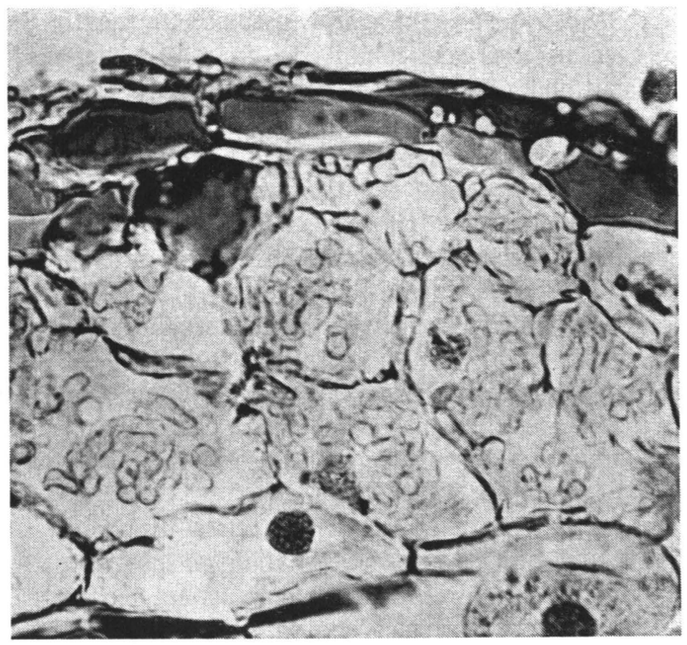


Fig. 5. Longitudinal section of an ectendotrophic pine mycorrhiza; nuclei of the cortical cells are visible. (From a greenhouse experiment of 1963.) × 650.

Isolation and Cultivation of the Fungus

Isolation

For isolating the fungal symbiont from mycorrhizae, the method of MELIN (1923a, 1936) was mainly used. After numerous failures, and after various substrates and methods for surface sterilization had been tested, the following procedure was adopted.

For isolation, thick young mycorrhizae with a smooth surface were selected. They were first washed in running water, and air bubbles then removed by immersing the roots for 30 sec. in 70 % alcohol. Surface sterilization was performed by treating the roots with 0.1 % HgCl or 30 % H_2O_2 for 5—10 sec., after which they were washed with sterile water and transferred to agar plates. The basic substrate was s.c. Hagem agar (0.5 g NH_4Cl, 0.5 g KH_2PO_4, 0.5 g $MgSO_4 . 7\ H_2O$, 1 ml 1 % $FeCl_3$, 5 g glucose, 5 g malt extract, 1 000 ml H_2O, 15 g agar), which is often used as a standard substrate for isolating and cultivating mycorrhizal fungi (MODESS 1941). Even on this substrate results were at first unsatisfactory: all that grew out from roots, if anything, was a dark-colored mycelium of *atrovirens* type, or bacteria. Therefore, various modifications of the method were tested: yeast extract, coconut milk, soil extract, and streptomycin were added to the substrate, and the agar was replaced by gelatin. Streptomycin (30 p.p.m.) proved necessary to inhibit the growth of bacteria. Both yeast extract and coconut milk (30 %) were advantageous for the growth of the true endophyte. Most of the isolates were obtained on Hagem gelatin substrate to which both yeast extract and streptomycin had been added.

The first ectendotrophic fungal symbiont was isolated in November, 1962. During subsequent months some 150 strains were isolated. Morphologically they were indistinguishable, although there were considerable differences in the rate of growth. On gelatin substrate hyphae usually grew out of the mycorrhizae in 7—20 days. Most strains were isolated from 2-year-old pine seedlings of Hyytiälä old nursery; some strains, however, were also isolated from pine seedlings of other nurseries, where the pine mycorrhizae were ectendotrophic. One exactly similar strain (E—57) was isolated from an ectotrophic spruce mycorrhiza from Hyytiälä old nursery. On grounds of morphological similarity, it is probable that all the isolates belong to the same species.

After isolation, the further cultivation of the ectendotrophic strains was easy. They grew well on Hagem agar and on other common substrates used for fungi. On Hagem agar the fungus also formed a loose cottony aerial mycelium, while only surface and submerged mycelia were formed on liquid and gelatin substrates.

The colony on Hagem agar is light brown. The aerial hyphae are coarse, and of variable thickness (4—9 μ), straight and septate. There are no clamp connections and no conidia or other reproductive bodies. The submerged hyphae are hyaline, septate, winding and branching; there are often clamydospore-like swellings up to 30 μ thick.

Since no sporophores or other reproductive organs of the fungus are known, it is unnamed and its position in the calssification of fungi unknown. No sporophores were found in the nursery beds where ectendotrophic mycorrhizae were observed.

Synthesis Experiments

Experiments under aseptic conditions

The identity of the isolated fungi with the true mycorrhiza formers can be confirmed, of course, only by synthesis experiments under aseptic conditions. Therefore, synthesis experiments were started immediately after isolation of the suspected symbiont, the technique of MELIN (1936) being used.

The first inoculations were made in January 1963 with strains isolated the preceding November. At the first sampling in March no mycorrhizae were found but mycelia grew profusely along the roots. In April, however, several mycorrhizae were found having the same structure as those from which the fungi had been isolated (Figs. 6—7).

The aseptic synthesis experiment of the winter and spring of 1963 comprised more than 100 flasks and 23 fungal strains. Owing to unfavorable experimental conditions the growth of seedlings and fungi was irregular, and some of the experiments failed owing to too high a temperature in the greenhouse. However, typical ectendotrophic mycorrhizae were formed by six fungal strains, and

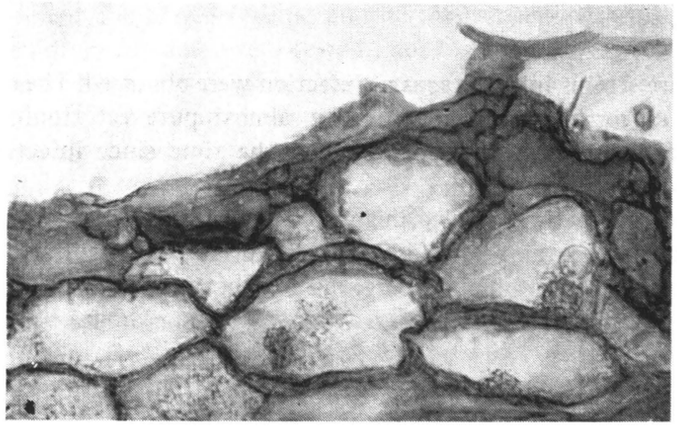


Fig. 6. Initial stage of the ectendotrophic infection. (From a synthesis experiment under aseptic conditions.)

Fig. 7. Advanced stage of the ectendotrophic infection. (From a synthesis experiment under aseptic conditions.)

with two more strains initial stages of infection were observed. The inner structure of the mycorrhizae ranged from an almost pure ectotrophic type to a heavy intracellular infection, depending on the time since infection. A thin mantle was usually present.

The synthesis experiments also included 15 spruce seedlings which were inoculated with seven strains. Mycorrhizae were formed with four strains. All the mycorrhizae were ectotrophic, although all four strains had been isolated from ectendotrophic mycorrhizae of pine.

Experiments under semiaseptic conditions

Experiments under semiaseptic conditions were started simultaneously with the above aseptic experiments. These experiments were conducted in open clay

pots, with sterilized soil and pure cultures of fungi. Soil of Hyytiälä old nursery, or a mixture of quartz sand and milled peat served as substrates. The substrates were moderately fertilized and sterilized in an autoclave. In the first experiment, which comprised some 50 pots, pots of 0.4 liter capacity were used.

Pine seeds were first sterilized with H_2O_2 and seeded in the pots on Feb. 7, 1963. Inoculation was performed on April 5. Seedlings were removed for examination for the first time on May 7 and then repeatedly until June 17.

Mycelia for inoculation were grown on MOSER's (1958) humus substrate, on which the isolated strains grew fairly well. For comparison, some pots were inoculated directly from Hagem agar plates. This experiment comprised 23 fungal strains, 16 of which were the same as were used in the experiments under aseptic conditions.

At the first sampling, on May 17, when seedlings inoculated with three strains were examined, inoculation proved successful. All the seedlings examined had mycorrhizae, mainly young stages with the fungus growing primarily as an intercellular net, but well-developed ectendotrophic mycorrhizae too. At the later samplings the majority of seedlings in the inoculated pots had ectendotrophic mycorrhizae. No other types of mycorrhizae were detected. All the control seedlings in uninoculated pots remained non-mycorrhizal, at least until June 17. Inoculation was as successful in both of the experimental soils, viz. sterilized nursery soil and the mixture of quartz sand and milled peat. The same type of ectendotrophic mycorrhizae were formed by 22 of the 23 strains, including E—57, which had been isolated from an ectotrophic spruce mycorrhiza in Hyytiälä old nursery.

Inoculation from agar plates was not so reliable; many pots inoculated from agar proved non-mycorrhizal, while in the others inoculation had resulted in ectendotrophic infection.

To supplement the above results, another experiment was set up in which various inoculation methods were tested and, for comparison, some other mycorrhizal and non-mycorrhizal fungi were included. 150 ml clay pots were used, and nursery soil (36 pots) and milled peat (two pots only) served as substrates. After autoclaving 10 seeds were sown in each pot and later on they were thinned to five seedlings per pot. Seeding was done on June 14, 1963, the pots were inoculated on July 27, the first samples were taken on August 30, and the experiment was discontinued on October 4 of the same year.

The primary object of the experiment was to study whether suspension of mycelia can be used for mycorrhizal inoculation. The suspension was prepared by putting several fungal colonies which had grown in Hagem solution, with a small amount of water, into a Bühler homogenizer; they were homogenized for 30 sec. and water was then added to make the suspension to up 120 ml. For inoculation, 20 ml of this suspension was used per pot.

The experiment comprised the following fungal species and strains:

E—57 (the ectendotrophic fungus, isolated from an ectotrophic spruce mycorrhiza), inoculated both in suspension and from humus substrate,
Boletus variegatus, both in suspension and from humus and agar substrates,
Cenococcum graniforme, in suspension only,
Collybia dryophila, from humus and agar substrates only,
Five strains of *Mycelium radicis atrovirens*, from humus and agar substrates.

The result were briefly as follows:

Inoculation with E—57 was successful with both methods. Mycorrhizal structures were detected even at the first examination, and at the end of the experiment all the seedlings were mycorrhizal. There were also some spruce seedlings in the pots; their mycorrhizae were ectotrophic.

Inoculation with *Boletus variegatus* failed. All the seedlings inoculated in different ways were non-mycorrhizal at the end of the experiment.

Inoculation with a mycelial suspension of *Cenococcum graniforme* failed as well.

None of the five strains of *Mycelium radicis atrovirens* formed mycorrhizae; instead, black hyphae grew profusely around the roots.

All the seedlings in the uninoculated control pots were non-mycorrhizal.

In another experiment which was conducted simultaneously and under the same conditions, inoculation was performed by using soil from a pot of the previous semiaseptic inoculation experiment (p. 17; the strain E—35 had been isolated from an ectendotrophic pine mycorrhiza, and mycorrhizae in the pot were of the same type). This inoculation also resulted in ectendotrophic infection of all the seedlings.

Accordingly, the ectendotrophic fungus is easy to inoculate in various ways under semiaseptic conditions. This makes it a suitable organism for experimental work and, therefore, in later experiments the semiaseptic technique was applied on a large scale.

Results of continued synthesis experiments under aseptic and semiaseptic conditions are reported by LAIHO (1965).

Pure Culture Experiments

The following pure culture experiments were aimed primarily at comparison of the ectendotrophic fungus with other mycorrhizal and non-mycorrhizal fungi of forest soil, the behavior of which in pure culture has been studied (e.g. MELIN 1925; MODESS 1941; LINDEBERG 1942; NORKRANS 1944, 1950; MIKOLA 1948; etc.).

The pH requirements of eleven strains of the ectendotrophic fungus were studied by the same technique and with the same nutrient solutions as were used by MODESS (1941) and LINDEBERG (1942). The different strains did not differ much in their relation to pH. Therefore, the mean values of the results with all eleven strains are presented in Fig. 8. At the same time the pH requirements of four strains of *Mycelium radicis atrovirens* were studied and the results are also shown in Fig. 8. It will be seen that in its relation to pH the ectendotrophic fungus resembles other mycorrhizal fungi of pine and spruce, of which the pH requirements are known (MODESS 1941), the total range being about 3.0—7.5 and the optimum round 5 and 6. On the other hand, *Mycelium radicis atrovirens* is largely independent of pH, as was already demonstrated by MELIN (1925); the fungus grew almost equally well over the whole pH range from 3 to 8.

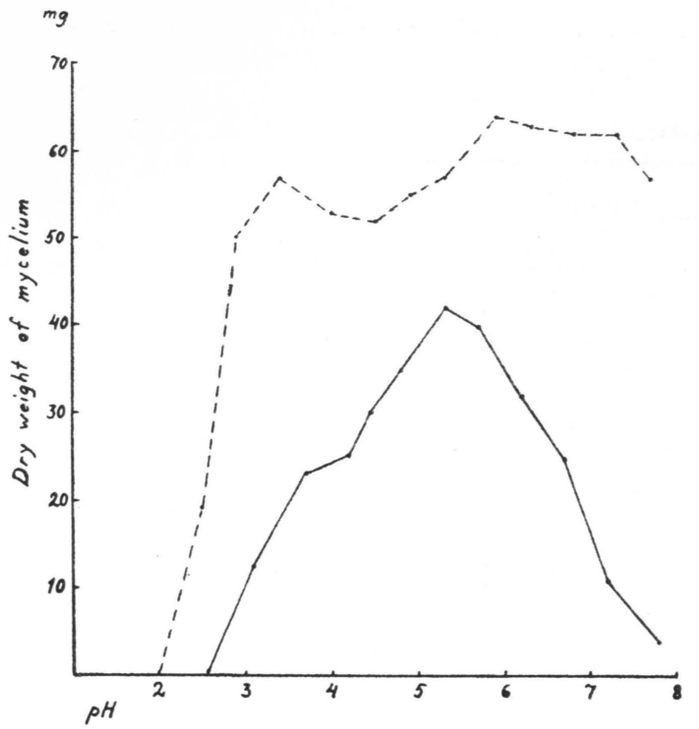


Fig. 8. The relation of the ectendotrophic fungus (solid line) and *Mycelium radicis atrovirens* (dotted line) to pH in liquid culture.

As is known from previous experiments (MELIN 1925; NORKRANS 1950; etc.), ectotrophic mycorrhizal fungi of forest trees prefer soluble carbohydrates as their carbon sources and, with few exceptions, are unable to utilize insoluble carbon compounds, such as lignin and cellulose, which, in turn, are easily utilized by many saprophytic soil fungi. The growth of some strains of the ectendotrophic fungus on different carbon sources is presented in Table 3.

Hagem solution without sugars was used as the substrate, and different carbon sources were added, the carbon contents corresponding to 0.5 g glucose per flask. Cellulose was given in the form of filter paper. Since the buffering capacity of the nutrient solutions was low, the pH generally dropped and therefore

growth soon ceased and the yield of dry matter remained low in spite of a fairly long incubation (25 days).

Table 3. Growth of some ectendotrophic strains on different carbon sources

Carbon source	Initial pH	Fungal strains									
		E—15		E—35		E—57		E—63		E—64	
		pH	mg	pH	mg	pH	mg	pH	mg	pH	mg
None	4.4	4.0	2	4.0	1	4.0	2	4.0	2	4.0	2
Glycerol	4.6	4.0	4	3.9	4	4.0	4	3.9	4	3.9	4
Mannitol	4.6	3.4	5	3.1	7	2.9	12	3.2	10	3.2	8
Citric acid	4.3	4.3	4	4.2	1	4.3	3	4.3	4	4.3	4
Glucose	4.6	3.2	9	2.8	15	2.8	16	3.0	13	3.0	12
Sucrose	4.6	3.2	11	2.9	12	2.8	17	3.0	12	3.0	14
Lactose	4.6	3.9	6	3.8	1	4.0	5	4.0	4	3.9	4
Maltose	4.8	3.8	4	3.7	3	3.8	5	3.8	5	3.9	4
Raffinose	4.6	3.2	8	3.0	8	2.9	10	2.9	8	3.0	9
Dextrin	4.7	3.7	5	4.2	2	3.6	4			3.6	6
Starch	5.0	4.4	4	4.2	2	4.1	4	4.4	5	4.2	5
1/4 glucose + 3/4 starch	4.6	3.1	13	2.9	12	2.9	14			3.0	14
Cellulose	4.2		0	4.0	0	4.0	0		0		0

Table 3 shows that in regard to carbon sources the ectendotrophic fungus is similar to many ectotrophic mycorrhizal fungi. Glucose and other simple sugars are usable, and also, to some extent, starch, glycerol, and mannitol, but no growth took place on cellulose. Different strains did not differ from each other. The fungus also grew faintly on autoclaved leaf litter; no sign of decomposition of cellulose or lignin could be detected, however.

The relation of the ectendotrophic fungus to different nitrogen sources is shown in Tables 4 a and 4 b. For comparison the latter experiment also included the known mycorrhizal fungus *Boletus variegatus* and three strains of *Mycelium radicis atrovirens*. The basic nutrient solution, to which different nitrogen sources were added, was as follows (MIKOLA 1948, p. 25):

Glucose	20 g	$FeCl_3$ (1 % solution)	0.5 ml
KH_2PO_4	0.35 g	$ZnSO_4 . 7\ H_2O$ »	0.5 »
K_2HPO_4	0.15 g	$MnSO_4 . 4\ H_2O$ »	0.5 »
$MgSO_4 . 7\ H_2O$	0.5 g	$CaCl_2$ (0.1 M solution)	5 »
Thiamin	50 γ	H_2O	995 »

Nitrogen sources were added at a rate corresponding to 0.3 g N per liter. Incubation lasted 29 (4 a) and 26 (4 b) days. Because some nutrient solutions were weakly buffered and the amounts of solution were small (12.5 ml per flask) the pH of some substrates rapidly became unfavorable, thus limiting the growth of the fungi. Hence, the steep change of the pH may

explain why the ectendotrophic strains grew much poorer on ammonium sulfate than on ammonium tartrate, for instance.

Table 4 a. Growth of some ectendotrophic strains on different nitrogen sources.

Nitrogen source	Initial pH	E—15		E—35		E—57	
		pH	mg	pH	mg	pH	mg
None	5.1	4.8	3	4.7	3	4.7	3
KNO_3	4.9	5.9	29	6.0	24	6.8	73
NH_4NO_3	4.9	3.0	24	3.0	19	2.8	24
$(NH_4)_2SO_4$	5.0	3.0	19	2.9	21	2.7	22
NH_4 tartrate	5.4	3.4	66	2.9	65	2.8	79
Glycine	5.0	4.6	9	4.6	9	4.4	12
Aspartic acid	3.2	3.0	1	3.0	5	3.0	7
Nucleic acid	3.4	3.2	6	3.2	6	3.1	5
Urea	6.6	6.6	3	6.6	2	6.4	2
Peptone	5.4	4.7	24	4.8	20	4.7	30
Hydrolyzed casein	5.1	4.6	15	4.6	16	4.6	34

Table 4 b. Growth of some ectendotrophic strains, *Mycelium radicis atrovirens*, and *Boletus variegatus* on different nitrogen sources.

Nitrogen source	Initial pH	Ectendotrophic strains								*Myc. radicis atrovirens*						*Boletus variegatus*	
		E—30		E—33		E—38		E—48		a		b		c			
		pH	mg	pH	mg	pH	mg	pH	mg	pH	mg	pH	mg	pH	mg	pH	mg
None	4.9	5.1	2	5.1	2	5.0	2	5.1	3	4.9	4	5.0	2	5.0	6	4.7	1
KNO_3	4.5	5.6	11	6.0	19	6.7	42	6.2	27	7.4	75	8.5	79	7.7	100	4.6	3
NH_4NO_3	4.5	3.4	14	3.2	17	3.0	18	3.1	20	3.7	96	5.4	95	4.4	100	2.7	60
$(NH_4)_2SO_4$	4.2	3.5	9	3.2	14	3.0	14	3.2	15	2.2	98	2.3	66	2.2	108	2.2	62
NH_4 tartrate	5.2	4.8	7	3.3	64	3.1	81	3.2	84	3.2	90	4.0	105	4.5	101	2.8	77
Glycine	4.7	4.8	5	4.8	8	4.7	10	4.9	7	4.0	73	4.0	65	3.8	115	4.6	10
Aspartic acid	3.2	3.2	1	3.2	3	3.1	3	3.2	3	5.6	125	5.5	103	4.6	112	3.5	57
Nucleic acid	3.3	3.4	2	3.4	2	3.2	3	3.4	4	3.2	112	3.1	125	3.0	124	3.4	1
Urea	6.2	7.1	3	6.6	38	7.1	6	7.2	6	5.0	98	5.2	103	4.4	100	6.4	8
Peptone	5.4	5.0	11	4.8	16	5.0	17	5.0	20	5.1	117	4.4	119	3.9	120	3.5	57
Hydrolyzed casein	5.6	5.4	18	5.6	14	5.4	12	5.6	15	5.8	116	5.3	119	5.2	127	3.5	78

As is seen from Tables 4 a and 4 b, ammonium salts were good nitrogen sources for the ectendotrophic fungus. The nitrate ion proved suitable too. Amino acids were utilized to some extent, while both peptone and casein hydrolyzate were good nitrogen sources. Slight differences can be noticed between different strains; thus, for instance, nitrate was more readily utilized by E—57 than by the other strains, and urea proved a good nitrogen source for E—33 only.

Accordingly, in their ability to utilize different nitrogen sources the ectendotrophic strains were very similar to *Boletus variegatus*, for instance, while *Mycelium radicis atrovirens* again proved more versatile.

Since the ectendotrophic fungus was noticed to be very common in forest nurseries where nowadays fungicides and herbicides are regularly applied, it was important to study whether the ectendotrophic fungus is more resistant to such chemicals than other mycorrhizal fungi which, in general, have proved more sensitive than saprophytic soil molds (LAIHO & MIKOLA 1964). Therefore an experiment was carried out by the same method as had been used by LAIHO and MIKOLA (l.c. p. 24—28) in pure culture experiments. Biocides were added to Hagem agar in different concentrations and the growth of different fungi on agar plates was measured.

The following chemicals were used: formalin (35 % formaldehyde), allyl alcohol, and Vapam (32.7 % Na methyldithiocarbamate, SMDC), and the following fungi: two ectendotrophic strains, the mycorrhizal fungi *Boletus variegatus* and *Cenococcum graniforme*, and *Mycelium radicis atrovirens*. The three last species were also included in the corresponding experiments of LAIHO and MIKOLA (1964).

Table 5. Relative growth of fungi on Hagem agar plates containing different concentrations of biocides.

Substance	Fungus	Concentration, %					
		0	0.0001	0.001	0.01	0.1	1.0
Formalin	*Boletus variegatus*	10		10	0	0	0
	Cenococcum graniforme ..	10	10	0	0	0	0
	E—15	10	8	9	0	0	0
	E—57	10	10	10	0	0	0
	Myc. radicis atrovirens	10	10	10	10	0	0
Allyl alcohol	*Boletus variegatus*	10		10	0	0	0
	Cenococcum graniforme ..	10		△	0	0	0
	E—15	10		10	6	4	0
	E—57	10	10	10	10	4	0
	Myc. radicis atrovirens	10	10	10	10	8	0
Vapam	*Boletus variegatus*	10		10	8	0	0
	Cenococcum graniforme ..	10		10	5	0	0
	E—15	10		8	7	△	0
	E—57	10		8	7	0	0

The results (Table 5) indicate that the ectendotrophic fungus is probably slightly more resistant, at least to allyl alcohol, than *Boletus variegatus* and *Cenococcum graniforme*. As regards Vapam, nursery experiments suggest the same (LAIHO & MIKOLA 1964, p. 20).

Furthermore, some preliminary experiments were conducted to study whether any antagonistic relations exist between the ectendotrophic fungus and other fungi. This was done by transferring two species of fungi, viz. the ectendotrophic fungus and some other species, to the same agar plate, 2 cm apart, and then observing the growth of the fungi towards each other. The ectendotrophic strain E—57 was grown together with the following 10 species: *Boletus bovinus*, *B. luteus*, *B. variegatus*, *Amanita muscaria*, *Lactarius rufus*, *Paxillus involutus*, *Laccaria laccata*, *Stropharia hornemannii*, *Cenococcum graniforme*, and *Mycelium radicis atrovirens*.

In most cases no stimulating or inhibiting effects were observed between the fungi; the colonies just grew out evenly in all directions until they touched each other and then continued one within or above the other. Only *Boletus bovinus*, *Paxillus involutus*, and *Mycelium radicis atrovirens* exerted a distinct, and *Boletus variegatus* probably also a slight, inhibiting influence on E—57. On the other hand, E—57 and *Laccaria laccata*, when growing together, promoted each other's growth.

These experiments, although only preliminary, indicate that the ectendotrophic fungus is no strong antagonist to other mycorrhizal fungi. Antagonism between fungi can hardly play any decisive role in determining which species in each particular case occurs as the mycorrhizal associate.

Occurrence of the Ectendotrophic Mycorrhiza in Finland

Forest Nurseries

As was stated previously, the dominance of the ectendotrophic mycorrhiza in Hyytiälä old nursery and its absence from the new one was the first thing that attracted attention. Therefore a survey was considered desirable to ascertain the commonness of the ectendotrophic fungus and the corresponding type of infection in Finnish nurseries in general.

The survey was conducted in September of 1962 by collecting samples (20—30 seedlings) of 1-year-old pine seedlings from 20 nurseries. Soil samples were also taken from the same nursery beds and analyzed, and data collected on the age of the respective nurseries, the fertilization, use of biocides, etc. Two samples were obtained from some nurseries, representing the old and new parts, and since the material was supplemented with data relating to those nurseries where investigations had already been conducted, the whole material comprised 34 sampling points in 24 nurseries located in different parts of Finland between the south coast and the arctic circle.

Ten seedlings of each sample were measured and the number and macroscopic appearance of mycorrhizae were recorded. The inner structure of the mycorrhizae was studied microscopically, different types of short roots of each sample being mounted in paraffin and sectioned.

The nursery survey revealed as follows:

— in 12 samples all the mycorrhizae were ectendotrophic;
— in 10 samples all the mycorrhizae were ectotrophic;
— in 12 samples both types of mycorrhizae were found.

When the presence or absence of ectendotrophic mycorrhizae were compared for soil properties, hardly any correlation could be noticed. In the nurseries where the ectendotrophic mycorrhiza was predominant, the soil pH was, on the average, slightly higher than in the others, as is indicated below; the difference was not consistent, however.

Samples with ectendotrophic mycorrhizae only:	pH 5.1 —6.6
» with ectotrophic mycorrhizae only:	pH 4.0[1]—6.2
» with both ectotrophic and ectendotrophic mycorrhizae:	pH 4.4 —6.1

Instead, a clear correlation prevailed between the age of the nursery and the type of mycorrhizae. All the samples with solely ectendotrophic mycorrhizae came from old nurseries where pine seedlings had been grown for several years, even for decades, while several samples devoid of ectendotrophic mycorrhizae were obtained from recently established nurseries where pine was being grown for the first time. The same phenomenon which drew attention in Hyytiälä nursery (mycorrhizae ectendotrophic in the old part of the nursery and ectotrophic in the new one) was observed in two other nurseries, while the opposite never occurred. However, there also was some old nurseries in which all the mycorrhizae were ectotrophic and which had probably escaped ectendotrophic infection.

The above nursery samples were used to investigate whether any relationship prevails between the size of the seedlings and the structure of the mycorrhizae. No correlation was detected, however. When two groups of nurseries were compared, in one of which all the mycorrhizae were ectendotrophic and in the other ectotrophic, the average size of the seedlings was approximately the same in the two groups. Likewise the macroscopic structure of the root system was determined by factors other than the mycorrhizal associate. When the development of root systems was measured by the percentage of dichotomous short root tips, great variation was observed, as is shown by the following figures:

[1] The lowest pH, 4.0, was in Nuojua Central Nursery of the Finnish Forest Service, which had been established on forest land. In the other nurseries of this group, which had been established on old agricultural land, the pH was above 4.9.

Samples with ectendotrophic mycorrhizae only:	2—30 %
» with ectotrophic mycorrhizae only:	0—29 %
» with both types of mycorrhizae:	0—27 %

In general, the percentage was lowest in the northernmost nurseries, where the seedlings were most behind in their development.

According to the above survey, conditions prevailing in forest nurseries are favorable for the ectendotrophic fungus and, therefore, the corresponding type of infection dominates in many nurseries. On the other hand, the fungus is probably lacking in ordinary agricultural soils and enters new nurseries in one way or another, most probably with transplants from other nurseries. This hypothesis was confirmed by the following experiments.

From Hyytiälä old nursery some 1-year-old pine seedlings were transferred to the new section and transplanted into beds of 1-year-old pines. Examination at the end of the growing season showed that the transplants had retained the ectendotrophic structure and the ectendotrophic infection also had spread to neighboring seedlings to a distance of 5—10 cm from the transplants.

Inoculation of the new nursery with »ectendotrophic soil» was also tried out. In seedling rows 5 cm deep holes were made in which soil of the old nursery was put. Examination three months later and further in the following summer revealed that the mycorrhizae at the inoculation points and their immediate neighborhood were ectendotrophic.

Natural Woodlands

The occurrence of the ectendotrophic mycorrhiza in natural woodlands was studied by taking root samples from mature and young pine stands from different parts of southern Finland; the samples were then mounted in paraffin, sectioned, stained, and examined under the microscope. Samples were taken from 75 stands of ages ranging from 15 to 250 years; they represented widely varying site conditions, from fertile mull soils to sandy barrens and *Sphagnum* bogs. No ectendotrophic mycorrhizae were found.

The mycorrhizal condition of young seedlings in natural habitats was studied by taking root samples from 63 localities. The age of the seedlings varied from 1 to 10 years, and seedlings originating from both natural reproduction and sowing were sampled. The sites represented all the natural habitats of pine and, in addition, both sowings and natural regeneration on old farmland were included. Most of the sampling points were either burnt or unburnt clear-cut areas, but seedlings growing under a dense tree canopy were also sampled. When possible, root samples of seedlings and mature trees were taken from the same stands or localities.

The great majority of the seedling mycorrhizae were ectotrophic, representing the same types as were present on mature trees. Ectendotrophic mycorrhizae were discovered in 12 samples and even then they formed a minority of all mycorrhizae.

Almost all of the ectendotrophic mycorrhizae were found on 1- to 3-year-old seedlings on burnt clear-cut areas. On unburnt soil they were found only twice, viz. on natural reproduction in an abandoned nursery (where during the nursery use ectendotrophic infection had probably been dominant) and on seedlings established by sowing on an old meadow. Sometimes a typical ectendotrophic infection was present in the long roots, while the short roots were ectotrophic mycorrhizae. Such a phenomenon also has been reported by Goss (1960, p. 24). Furthermore, in some of the mycorrhizae the old basal part was ectendotrophic, without a mantle, while the young part was ectotrophic and had a mantle (cf. pp. 27—32).

In another study, when the effect of prescribed burning on the commencement of mycorrhizal infection was investigated (Mikola et al. 1964), ectendotrophic mycorrhizae were found on 1-year-old pine seedlings only on a heavily burnt area.

Inoculation of forest soil with the ectendotrophic fungus is possible under certain conditions, as is shown by the following experiment. On an area which had been broadcast burnt in the spring of 1962 and seeded in patches on June 12, a small amount of soil from Hyytiälä old nursery was added to some seeding patches on June 21. Examination in October revealed that the seedlings in those patches where nursery soil had been added had ectendotrophic mycorrhizae, while seedlings in other patches had ectotrophic mycorrhizae only. Even the following summer (July 12) ectendotrophic infection was still present in the inoculated patches.

Soil inoculation was also tried in some mature stands by removing the humus layer and placing »ectendotrophic» nursery soil in contact with pine roots. This treatment, however, had no effect on the structure of the mycorrhizae of mature trees.

Transplanting of Seedlings from the Nursery into the Field

The above review clearly shows that there is a great difference in mycorrhizal relations between nurseries and natural forest lands. In nurseries the ectendotrophic mycorrhiza is the commonest, and sometimes even the only type of mycorrhiza, while in forest soil it is seldom found. Whenever ectendotrophic mycorrhizae were found in forest soil they always belonged to young seedlings; in the roots of mature trees typical ectendotrophic infection was never detected. The survey conducted by Laiho (1965) in nurseries and forest stands led him to a similar conclusion.

Previous observations on ectendotrophic mycorrhizae have also been made mainly in forest nurseries. Björkman (1942), who described ectendotrophic mycorrhizae in some natural forest soils, likewise found them only in young plants, particularly in stunted seedlings with slow growth, while no mention is made of their occurrence in older trees. Some reports on ectendotrophic mycorrhizae are based on pot experiments in greenhouses (e.g. Bergemann 1955), i.e. on seedlings 1—2 years old. Likewise the treatise of Goss (1960) is primarily based on nursery and pot experiments. Furthermore, one may notice that in a greenhouse experiment (Goss 1960, Table 2) the ectendotrophic mycorrhiza was the sole type in nursery soil, while in other soils either ectotrophic mycorrhizae alone or both types were present.

As was stated before, the particular type of ectendotrophic mycorrhiza which is the object of this paper is probably formed by a specific fungus, which predominates in many nurseries but plays an insignificant role in natural forest soils. Therefore, it was important to trace the changes taking place in mycorrhizal structures when ectendotrophically mycorrhizal pine seedlings are transplanted from the nursery into field conditions.

Such experiments were conducted in three summers (1961—1963) by transplanting seedlings from Hyytiälä old nursery to different forest sites. To prevent damage to the mycorrhizae, the seedlings were planted immediately after lifting, i.e. in less than two hours. In each locality 20 or 30 seedlings were planted in the spring, and they were removed for examination, one at a time, in the same and the following summer.

Then the main emphasis was put on the following questions:

1) how did the old mycorrhizae develop which continued growth after transplantation;
2) of what type were the young mycorrhizae formed in the new habitat.

At microscopic examination the following structural types were distinguished:

a_1 Continued ectendotrophic growth.
a_2 »Change», the old part being ectendotrophic and the young one ectotrophic.
b_1 Young ectendotrophic mycorrhizae.
b_2 Young ectotrophic mycorrhizae.

In the spring of 1961 1-year-old pine seedlings were transplanted into six localities, viz:

1—2. Open pine stands on dry sandy soil (*Calluna* type).
3. An open, old (200 y.) pine stand on rocky ground, with lichen and dwarf-shrub vegetation.
4. A clear-cut area of a medium site.
5. D:o, burned 5 years before.
6. A wet depression, with peat soil and swamp vegetation, on the above clear-cut area.

Samples were taken in July and October of 1961 and in the summers of 1962 and 1963.

Likewise in the spring of 1962 1-year-old pine seedlings were transplanted to the same areas and, in addition, to the following localities:

7. A dense spruce stand on a medium site (*Hylocomium-Vaccinium myrtillus* vegetation).
8. An adjoining area of the same site, clear-cut 2 years ago.
9. D:o, burned in the spring of 1962.
10—11. Abandoned fields.

Samples were taken a few times during the summers of 1962 and 1963.

Some transplanting was still done in the spring of 1963 when 2-year-old pine seedlings were planted in the above areas 1, 3, 5, and 7, and, in addition, to six other areas. Samples were taken in July and September of the same year.

Altogether more than 500 mycorrhizae were sectioned and examined under the microscope. The results are briefly as follows.

Mycorrhizae generally survived the transplanting and continued to grow in the new habitat. A change of the mycorrhizal structure usually took place, however, immediately after transplanting; i.e. the young growth formed in the new habitat was ectotrophic while the ectendotrophic structure remained in the old part. The young ectotrophic part usually had a distinct mantle, while in the old part a mantle was lacking. Correspondingly, the old and new parts were often of different colors.

In other cases, however, the ectendotrophic infection continued to the young part of a mycorrhiza, sometimes for a short time only and sometimes even throughout the whole summer.

The change of the mycorrhizal structure took place in various ways. In most cases the change was sharp, i.e. after transplantation the ectendotrophic fungus did not enter the new growth, which was immediately infected by some other fungus forming a mantle and Hartig net. Sometimes the ectendotrophic infection continued to the young part where the change took place gradually, i.e. for some time there had been two competing fungi, the ectotrophic one gradually gaining the dominance. Dichotomous mycorrhizae were also found, having one branch ectotrophic and the other ectendotrophic. Furthermore, quite commonly there was a short non-mycorrhizal zone between the ectendotrophic and ectotrophic parts of a mycorrhiza. Figs. 9—12 show some examples of changes.

The change of the mycorrhizal structure clearly depended on the site. On the abandoned fields (10 and 11) the change was fairly slow; old mycorrhizae usually continued their growth, retaining the ectendotrophic structure, and new ectendotrophic mycorrhizae were even formed. In the second summer, however, the new growth was usually ectotrophic, although some new ectendotrophic mycorrhizae still were formed. On the other hand, the change of the mycorrhizal structure was most rapid in natural forest soils of medium fertility, both under the

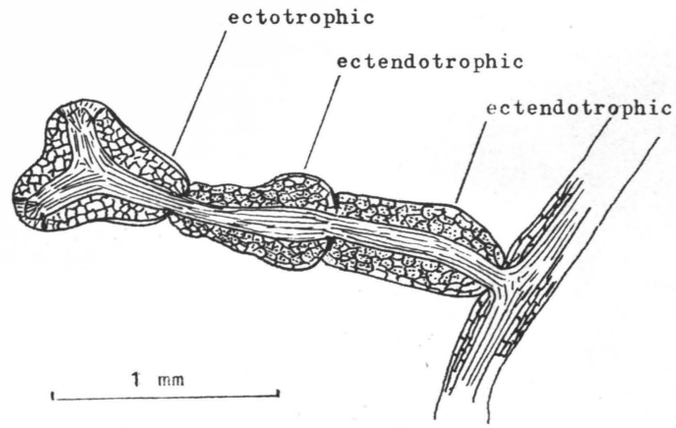


Fig. 9. A pine mycorrhiza with three distinguishable zones, viz. (1) the basal ectendotrophic zone, grown in the nursery in 1961, (2) the central ectendotrophic zone, probably grown in the nursery in the spring of 1962 before transplanting, and (3) the apical ectotrophic zone with a mantle, grown in the field after transplanting. The seedling was transplanted on May 21, 1962, from Hyytiälä old nursery into forest soil and removed for examination on August 27.

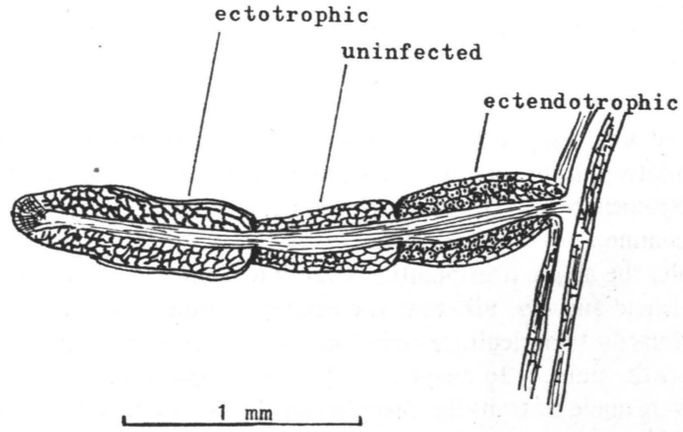


Fig. 10. Three distinguishable zones in a pine mycorrhiza, viz. (1) the basal ectendotrophic zone, grown in the nursery in 1961, (2) the central zone without any infection, probably grown in forest soil after transplanting, and (3) the apical ectotrophic zone, grown later the same summer. The seedling was transplanted from Hyytiälä old nursery into forest soil on May 24, 1962, and removed for examination on August 27.

tree stand and on fresh clearings (3, 7, 8, and several experimental plantings of 1963), where all the new growth of old mycorrhizae and all new mycorrhizae were ectotrophic. The change was somewhat slower on burnt areas where even the field survey (pp. 25—26) revealed some ectendotrophic mycorrhizae. Likewise in a very barren sandy soil (1 and 2) the change was slow; the ectendotrophic

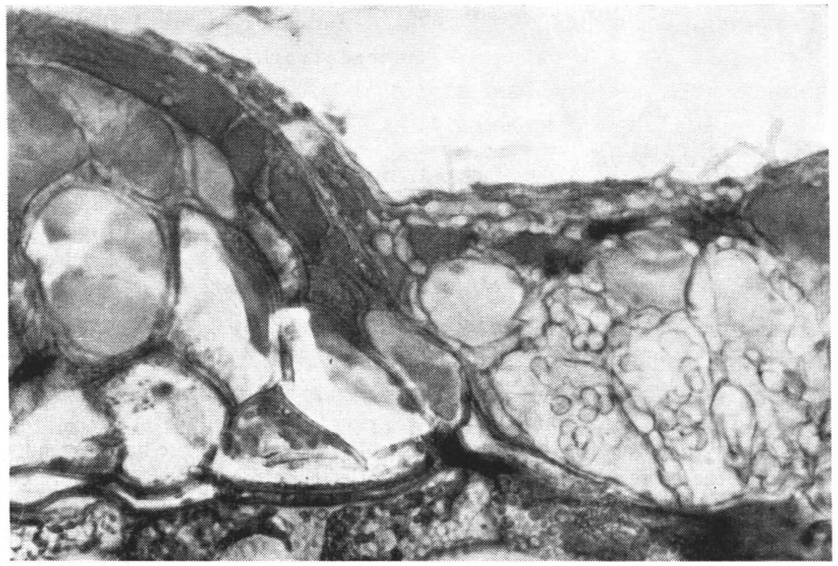


Fig. 11. A sharp boundary line between the ectendotrophic (right) and ectotrophic (left) parts of a pine mycorrhiza. The seedling was transplanted from Hyytiälä old nursery into forest soil (loc. 1) on May 24, 1962, and removed for examination on Oct. 15.

structure had not disappeared completely after three years, and some quite young ectendotrophic mycorrhizae were found even two years after transplanting. Similarly, BJÖRKMAN (1942) noticed that on such sites the ectendotrophic type was fairly common.

As a whole, the above transplanting experiments confirmed the results of the nursery and field surveys, viz. that the ectendotrophic mycorrhizal infection is confined primarily to agricultural soils and disappears when seedlings are transplanted from the nursery to forest soils. For additional information, further experiments were made by transplanting pine seedlings from forest soil back into the nursery. Such plantings were made in the springs of 1962 and 1963 by returning to Hyytiälä old nursery from areas 1, 3, 5, 6, and 8 the same seedlings which one year previously had been transplanted from the same nursery. Sampling a few months after replanting revealed that the seedlings had mainly retained the ectotrophic mycorrhizal structure. However, some reversions to the ectendotrophic structure were discovered. Likewise, the young mycorrhizae were largely ectotrophic, even though some young ectendotrophic mycorrhizae were found.

Accordingly, restoration of ectendotrophic structure, if it occurred, was slow and incomplete. On the contrary, change of mycorrhizal structure from ectendotrophic to ectotrophic took place in Hyytiälä old nursery too. This was noticed in the fall of 1964, when roots of 3-year-old pine seedlings were examined. Then

a

b

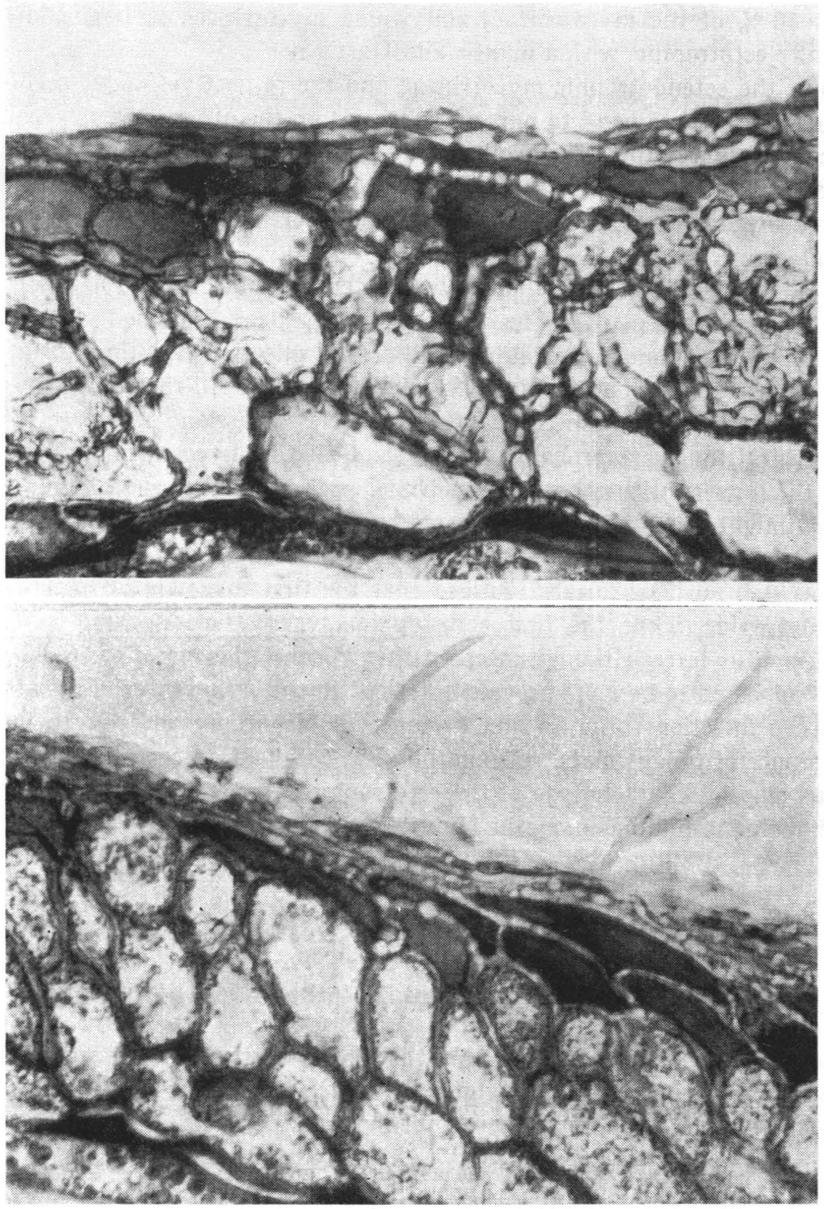


Fig. 12. Sections of the ectendotrophic (a) and ectotrophic (b) parts of the same mycorrhiza. The seedling was transplanted from Hyytiälä old nursery into forest soil (loc. 1) on May 24, 1962, and removed for examination on Oct. 15. Obs. clamp connections on the mantle of the ectotrophic part.

about 10 % of the mycorrhizae, and young mycorrhizae in particular, were typically ectotrophic, with a mantle and Hartig net.

Thus, the ectendotrophic mycorrhizae and the respective fungus seem to be confined on the one hand to nursery soils, and on the other to young seedlings. In the field survey (pp. 25—26) they were found on young seedlings only, never on mature trees. Likewise BJÖRKMAN (1942, pp. 20, 25) described ectendotrophic mycorrhizae on seedlings only and never mentioned their occurrence on full-grown trees.

In all probability Goss (1960) found ectendotrophic mycorrhizae on older trees as well (l.c. p. 41: »The types of mycorrhizae described in this study were found in common association in all of the collections from nursery sites to stands 25 to 50 years old on a wide variety of soils.»). All the stands, however, were plantations established on formerly treeless grassland where ectotrophic mycorrhizal fungi are probably lacking and all the mycorrhiza formers came from the nursery with the seedlings. Under such conditions the ectendotrophic fungus might have a better change to survive, although even in the stands studied by Goss ectotrophic mycorrhizae formed the great majority (l.c. p. 7).

Goss (l.c. pp. 23—25) also noticed that the first mycorrhizal infection was ectendotrophic, while the first ectotrophic mycorrhizae appeared as late as three months later, although later on they formed the majority. (»The latter type[1] appeared to be more prevalent at least in the early stages of growth with extensive infections prior to any evidence of dichotomy and long before the appearance of fungus mats, rhizomorphs and coralloid mycorrhizae with white fungus mantles characteristic of the ectotrophic types.») This is in good agreement with the findings made in Hyytiälä old nursery.

Ecology of the Ectendotrophic Mycorrhiza

Experimental Methods

To supplement the information on the ecology of the ectendotrophic mycorrhiza and the respective fungus which can be drawn from the above observations and experiments, some nursery and greenhouse experiments were conducted.

A preliminary nursery experiment was arranged in 1962 in Hyytiälä old nursery. The experiment included 47 soil treatments (88 plots of 2 × 1 m), viz. in different combinations and concentrations:

a. Acidification with sulfur and H_2SO_4 (pH down to 3.6)
b. Liming (pH up to 7.6)
c. Fertilization with N, P, and K
d. Addition of organic matter (peat and forest humus).

[1] ectendotrophic

Thus the experiment was conducted in a nursery soil containing both ectotrophic and ectendotrophic mycorrhizal fungi, the ectendotrophic mycorrhiza being the predominant type. The experiment was aimed primarily at revealing whether some of the above treatments could change ecological conditions to favor the ectotrophic type of fungi or how such structural details of the ectendotrophic mycorrhiza as the heaviness of the intracellular infection, are influenced by environmental factors.

Seeding was done in June, 1962. Because of unfavorable weather conditions germination and growth were slow and the first root samples for microscopic examination were not taken until October. More samples were taken the following summer.

This preliminary experiment, however, failed to provide an answer to the question. In the control plots all the mycorrhizae were ectendotrophic, but so they were in differently treated plots too. Some slight mycorrhizal differences, however, were detectable, which might have been due to the treatments, an assumption which could be checked by comparison with the results of the greenhouse experiments.

The first greenhouse experiment was also started in summer 1962. Soil was taken in clay pots from Hyytiälä old nursery and was not sterilized. The treatments of acidification, liming, and fertilization were the same as in the nursery experiment (addition of humus was not included). Seeding was done in July, and the seedlings were harvested for examination the following January. The results were consistent with the nursery experiment.

Greenhouse experiments were continued in the summer of 1963 on a larger scale with another technique. Since the ectendotrophic fungus had been isolated the preceding fall (cf. p. 14) and its inoculation under semiaseptic conditions had proved easy and successful (pp. 16—18) sterilized soil and artificial inoculation were used in further experiments.

In most of the experiments soil of Hyytiälä old nursery and milled peat served as substrates, and in addition, ordinary sand, pure quartz sand, and various types of forest humus were used in some experiments. Clay pots of 150 ml were used. Seeding in the pots was done on June 11—12, and inoculation with pure cultures of fungi was performed on July 23—26. A sufficient number of control pots was left uninoculated, of course. Three strains of the ectendotrophic fungus were used for inoculation, viz. E—15 (the first isolate), E—35 (isolated from an ectendotrophic pine mycorrhiza), and E—57 (isolated from an ectotrophic spruce mycorrhiza, Hyytiälä old nursery). Mycelia for inoculation were grown on a humus-nutrient solution substrate (MOSER 1958).

The first root samples were taken on August 27—30, when the pots were thinned to 5 seedlings per pot. If dichotomous short roots were found they were fixed for microscopic examination.

The experiments were discontinued on October 4—6. All the seedlings were removed, the dry weights of shoots and roots were determined, the number of short root tips was counted, the tips of dichotomous short roots separately, and various types of short roots were fixed. The soil pH of the pots also was determined at the end of the experiment.

The experiments surpassed all expectations, in that all the seedlings in the uninoculated pots were non-mycorrhizal whilst those in the inoculated pots were without exception mycorrhizal. The structure and development of the mycorrhizae bore no relation to the fungal strains, i.e. all three strains were equally infective and produced similar mycorrhizae. No other mycorrhizae were present, — in other words, the pots had escaped an outside infection surprisingly well.

Mycorrhizae and uninfected short roots could be distinguished by microscopic examination only. The color of the two types of short roots was the same, and the difference in thickness small (Table 2).

The only conspicuous macroscopic difference between inoculated and uninoculated seedlings was in the dichotomy of the short roots; the inoculated seedlings had always a great

number of dichotomous short roots. True, uninoculated seedlings also had some dichotomous short roots, and thus the dichotomy was no reliable indication of mycorrhizal infection, as was the case in some field experiments (MIKOLA et al. 1964). The fungal infection, however, strongly promoted the dichotomous forking of short roots. Uninoculated seedlings usually had dichotomous short roots, if any, with two branches only, while inoculated seedlings often had coralloid short roots with 4, 8, and even more branches. At the first sampling (at the end of August) dichotomous short roots were found on inoculated seedlings only. Mycorrhizal infection was usually, however, already present before forking.

The factors studied in these greenhouse experiments with sterilized soil and pure culture inoculation were primarily the same as in the above nursery experiment, viz. the effects of pH, fertilization, and the proportion of humus and mineral soil, and in addition, the effect of light. The experiments comprised 344 pots, 102 of them being uninoculated controls, with 5 seedlings in each pot.

Some supplementary greenhouse experiments with non-sterilized soil and without inoculation were also conducted in 1963.

The individual experiments are reported in the following, in connection with the ecological factors studied.

Light

As has been shown by BJÖRKMAN (1942, 1949) and others, light exerts an important influence on the formation of ectotrophic mycorrhizae. According to BJÖRKMAN, the fungus penetrates the root and a symbiosis is established only if the roots contain a certain surplus of soluble carbohydrates, which, in turn, depends on the light conditions. Several later investigations have confirmed this carbohydrate theory of BJÖRKMAN's (cf. BOULLARD 1963). Further, some findings indicate that not all mycorrhizal fungi are equally dependent on the light received by the host and, therefore, reduction of light may favor certain fungi and mycorrhizal types and suppress others (HARLEY & WAID 1955); for instance, *Cenococcum graniforme* is able to form mycorrhizae at a lower light intensity than many other fungi (MIKOLA 1948). Therefore, a study of the light requirements of the ectendotrophic mycorrhiza was also thought desirable.

The light intensity was regulated with two lath screens, the light under the screens being 20 % and 2 % of the full light outside the greenhouse. The light in the greenhouse was 60 % of the full light. The shading was started on July 9, i.e. four weeks after seeding and two weeks before inoculation. The strains E—35 and E—57 were used as inocula. Each treatment comprised two replicate pots; thus, the figures in Table 6 represent the averages of 10 seedlings.

Table 6 shows that, as was stated before, inoculation strongly promoted the dichotomous forking of short roots. Further, the table shows that shading reduces root growth especially. Thus, lowering the light from 60 % to 20 % reduced the root weight by about 50 %, while the effect on shoot growth was much smaller. The effect of shading on the number of short roots was still more pronounced. At 2 % light the seedlings still survived, but hardly any root

growth took place. Consequently, the top/root ratio at 60 % light was 1.3, at 20 % light 2.5, on the average, and at 2 % light between 5 and 6.

Mycorrhizal relations. As in all the experiments of 1963 with sterilized soil, the short roots of uninoculated seedlings remained without mycorrhizal or pseudomycorrhizal infection. On the inoculated seedlings, both in peat and in nursery soil, all the short roots were ectendotrophically mycorrhizal both at 60 % and at 20 % light. The structure of the mycorrhizae was also very much the same at both light intensities. Perhaps at 20 % light E—35 grew more intercellularly and showed a stronger tendency to mantle formation than at 60 % light. At 2 % light the seedlings had only a few short roots and no dichotomous ones, and no true mycorrhizae were formed. Some slight indication of fungal infection was detectable, however. Thus, both E—35 and E—57 occasionally penetrated between the cortical cells and locally formed a rudimentary net; likewise a thin mantle was locally present and even intracellular hyphae were observed inside some cortical cells.

The above observation suggests that the ectendotrophic mycorrhizal fungus is fairly independent of the light received by the host. The infection took place at the same rate and the mycorrhizae were structurally similar at the light intensities of 60 % and 20 %, and even at as low an intensity as 2 % the fungus still showed some tendency to form mycorrhizal structures.

Table 6. Dry weight and root development of pine seedlings under different light intensities with and without fungal inoculation.

Soil	Light, % of full light	Control					Inoculated									
							E—35					E—57				
		Dry weight mg		No. of short root tips	Tips of dichotomous short roots		Dry weight mg		No. of short root tips	Tips of dichotomous short roots		Dry weight mg		No. of short root tips	Tips of dichotomous short roots	
		Shoot	Root		No.	%	Shoot	Root		No.	%	Shoot	Root		No.	%
Nursery soil ..	60	35.6	26.4	251	2	1	36.1	27.1	227	98	43	35.6	26.5	253	78	31
	20	32.7	15.2	136	3	2	30.7	11.1	86	16	19	38.2	16.0	123	21	17
	2	8.5	1.8	7	—	—	8.8	1.4	8	—	—	10.7	1.8	8	—	—
Peat ..	60	35.7	28.5	325	9	3	31.8	24.0	211	42	20	36.4	27.2	255	45	18
	20	28.2	14.7	124	11	9	22.4	8.9	77	18	13	25.9	8.8	95	15	16
	2	7.4	1.2	3	—	—	6.5	1.2	4	—	—	6.9	1.2	4	—	—

Some earlier observations also support the hypothesis of the lower light requirements of the ectendotrophic mycorrhiza. According to BJÖRKMAN (1942, p. 20) this type of mycorrhiza is common on pine seedlings under a dense canopy (»vor allem bei Pflanzen geschlossener Bestände»). The same reason may explain the particularly common occurrence of the ectendotrophic mycorrhizae on

young seedlings, which, having only cotyledons and a few juvenile needles, must have a fairly low photosynthetic capacity. For all that, in the above experiment, when inoculation was performed as early as six weeks after seeding, the mycorrhizal infection advanced quite rapidly, as examination four weeks later confirmed. Likewise Goss (1960, p. 24) noticed that 2-month-old pine seedlings had numerous ectendotrophic mycorrhizae, while the first ectotrophic mycorrhizae were not detected until 3 months later. With advancing age the ectotrophic type gains more dominance, this fact indicating that an increased photosynthetic capacity probably favors the ectotrophic fungi (cf. pp. 28—30).

Hydrogen Ion Concentration

When reasons for the common presence of the ectendotrophic mycorrhiza in forest nurseries and its rare occurrence in natural forest soils are discussed, the soil acidity must also be consedered as an ecological factor. Since most Finnish nurseries have been established on agricultural soils and liming is also often practised, the pH in nursery soils is usually considerably higher than in forest soils. The nursery survey (pp. 23—25) indicated that in nurseries where the ectendotrophic mycorrhiza was dominant, the pH of the soil was, on the average, higher than in those nurseries, in which the mycorrhizae were ectotrophic, even though the difference was fairly small and not always consistent. Some literature references likewise suggest promotion of the ectendotrophic mycorrhiza by soil alkalinity. Thus, in one of Goss's (1960, p. 26) experiments the ectendotrophic mycorrhiza was the only type in a nursery soil with a high pH (7.1), while in the other soils, where the two types or the ectotrophic one alone were present, the pH was lower. According to LEVISOHN (1954), the »haustorial infection» is characteristic of »nursery soils of agricultural type with a high or moderately high pH». Likewise, in a greenhouse experiment made by BJÖRKMAN (1942, p. 124), in a limed peat soil (pH 6—7) all the mycorrhizae were ectendotrophic, while in the same soil without liming they were ectotrophic. On the other hand, the natural forest soils in which BJÖRKMAN found ectendotrophic mycorrhizae were remarkably acid (pH $\sim$ 4.0). The above pure culture experiments (p. 19) showed that the pH requirements of the ectendotrophic fungus are very much the same as those of the common mycorrhizal fungi of forest soils.

In the pot experiment of 1963, autoclaved nursery soil and peat served as substrates. The pH was regulated by addition of H_2SO_4, $CaCO_3$, and $Ca(OH)_2$. (The initial pH's 5.3 and 4.4 represent the experimental soils without addition of acid or lime.) The strains E—35 and E—57 were used as inocula. The controls included only one pot (5 seedlings) per treatment, and the inoculated series two pots. The development of the seedlings and their roots systems is presented in Table 7.

As is seen in Table 7, the pH did not remain constant but turned towards neutrality from both the acid and alkaline range. However, the whole range of the pH was fairly wide even at the end of the summer. The pine grew quite well over the whole pH range of the experiment, except at the highest and lowest values.

In this experiment, as in all the others, the effect of fungal inoculation on the dichotomy of the short roots is apparent. A striking exception is the series »nursery soil, pH 5.3», where even uninoculated seedlings had remarkably numerous dichotomous short roots. Since there was only one inoculated pot, an outside infection was suspected. Contamination is improbable, however, for a thorough microscopic examination did not reveal any mycorrhizal infection in this pot or in the other uninoculated pots. The reasons for the profuse dichotomy of short roots in this particular pot (also to some extent in the uninoculated »peat, pH 12.6») are unknown. Even in this pot, however, almost all the dichotomous short roots had two branches only, while in the corresponding inoculated pots there were numerous coralloid mycorrhizae with 4 or more branches. It should be noted that corresponding exceptions did not occur in any other pot experiments of 1963.

Table 7. Dry weight and root development of pine seedlings grown in sterilized soils at different pH levels with and without fungal inoculation.

Soil	pH		Control					Inoculated								
								E—35					E—57			
	Initial	Final	Dry weight mg		No. of short root tips	Tips of dichotomous short roots		Dry weight mg		No. of short root tips	Tips of dichotomous short roots		Dry weight mg		No. of short root tips	Tips of dichotomous short roots	
			Shoot	Root		No.	%	Shoot	Root		No.	%	Shoot	Root		No.	%
Nursery soil ..	1.7	3.6	10.3	6.3	20	1	(5)	7.0	4.5	11	—	—	8.5	9.5	55	2	(4)
	2.1	3.7	21.6	19.7	162	22	14	21.2	17.0	152	53	35	27.3	24.5	205	48	23
	5.3	5.5	49.2	40.2	258	116	45	43.9	29.7	196	94	48	44.2	24.6	200	63	32
	6.7	7.2	32.4	24.7	245	1	△	36.2	26.3	202	87	43	35.6	27.9	286	81	28
	7.0	7.7	38.8	28.1	258	2	1	36.0	27.9	252	108	43	35.7	25.2	220	75	34
	7.6	7.7	42.0	24.8	258	14	5	35.9	26.0	196	82	42	34.3	25.1	254	102	40
	11.5	8.1	33.0	18.2	129	1	1	32.5	22.0	146	38	26	30.3	17.1	130	44	34
	12.2	8.2	22.3	14.6	86	2	2	22.9	12.5	59	6	10	23.9	13.4	83	24	29
Peat ..	2.2	3.6	25.0	16.5	118	14	12	25.2	23.0	190	39	21	27.6	27.1	196	33	17
	2.5	4.0	31.6	24.4	168	7	4	26.2	23.4	221	41	19	31.4	28.3	263	57	21
	4.4	4.3	31.8	30.5	248	4	2	38.2	33.2	293	61	21	36.2	31.3	339	53	16
	6.0	6.9	29.8	28.0	273	7	3	33.4	25.5	228	42	18	29.2	21.6	244	33	14
	7.3	7.6	41.6	29.1	378	11	3	29.6	22.6	194	41	21	43.6	32.8	265	58	22
	9.2	7.8	34.2	27.0	219	2	1	34.0	23.5	229	67	29	33.2	29.1	265	62	23
	11.3	7.6	25.2	27.2	161	—	—	28.2	22.2	163	46	28	30.7	22.0	212	56	26
	12.6	11.9	19.3	11.8	70	25	36	23.3	12.9	99	47	47	22.0	12.9	116	43	37

Mycorrhizal relations. All the inoculated pots contained numerous mycorrhizae, with the sole exception of »nursery soil, pH 1.7», where the fungus had probably died immediately after inoculation. At the time of harvesting, all the short roots examined, both dichotomous and single, were mycorrhizal. The structure of the mycorrhizae was always the same, ectendotrophic, and the intracellular infection was equally strong over the whole pH range from 3 to above 8 (Fig. 13). The rate of infection apparently did not depend on the pH, for at the first examination (Aug. 27) dichotomous short roots with ectendotrophic infection were found in all the other inoculated pots except in »nursery soil, pH 1.7», while no dichotomous short root was found at that time in uninoculated pots.

It is noteworthy that the ectendotrophic fungus was able to infect the seedlings and to form mycorrhizae even at alkalinities higher than pH 10, while in pure culture the upper limit of its pH range is round 8 (Fig. 8). This shows that pure culture experiments in liquid substrates are not always applicable to natural conditions.

Accordingly, the ectendotrophic fungus can form mycorrhizae over a wide pH range, at least if no other mycorrhizal fungi are present, and the structure of the mycorrhizae is fairly independent of the soil pH. Another question is whether the pH of the environment has any influence on the competitive capacity of the ectendotrophic fungus. This problem can be discussed in the light of the nursery and greenhouse experiments in which the soil was not sterilized.

A greenhouse experiment with unsterilized soil was conducted simultaneously with the above experiment and under the same conditions, with the exception that the pots were considerably larger, of 3.7 liter capacity. The soil had been taken from Hyytiälä old nursery, and the pH was regulated with lime and sulfuric acid. The main results are given in Table 8. As is seen, the seedlings grew bigger than in sterilized soil, probably owing to the larger size of the pots. It can also be noticed that pine seedlings again grew well over a fairly wide pH range, the optimum pH being from 4 to 6.

Again in this experiment, pH affected the structure of the mycorrhizae very little. At both ends of the experimental range (at the final pH's 3.3 and 11.4) the short roots examined were non-mycorrhizal; in all the other pots, however, with the final pH varying from 3.9 to 8.0, almost similar ectendotrophic infection was present. The dichotomous forking of short roots was most pronounced in a fairly acid soil (pH 4.0—5.5). Further, at the same pH range the mycorrhizae usually had a distinct mantle (up to 10 μ thick) while at a higher pH the mantle was very thin or lacking. Likewise, at higher pH (final 8.0) the commencement of mycorrhizal development was somewhat delayed.

Similarly, the nursery experiment (p. 33) did not reveal any clear correlation between the soil pH and the ectendotrophic mycorrhiza. It was noted, however, that seedlings in the acidified plots had both ectotrophic and ectendotrophic

a

b

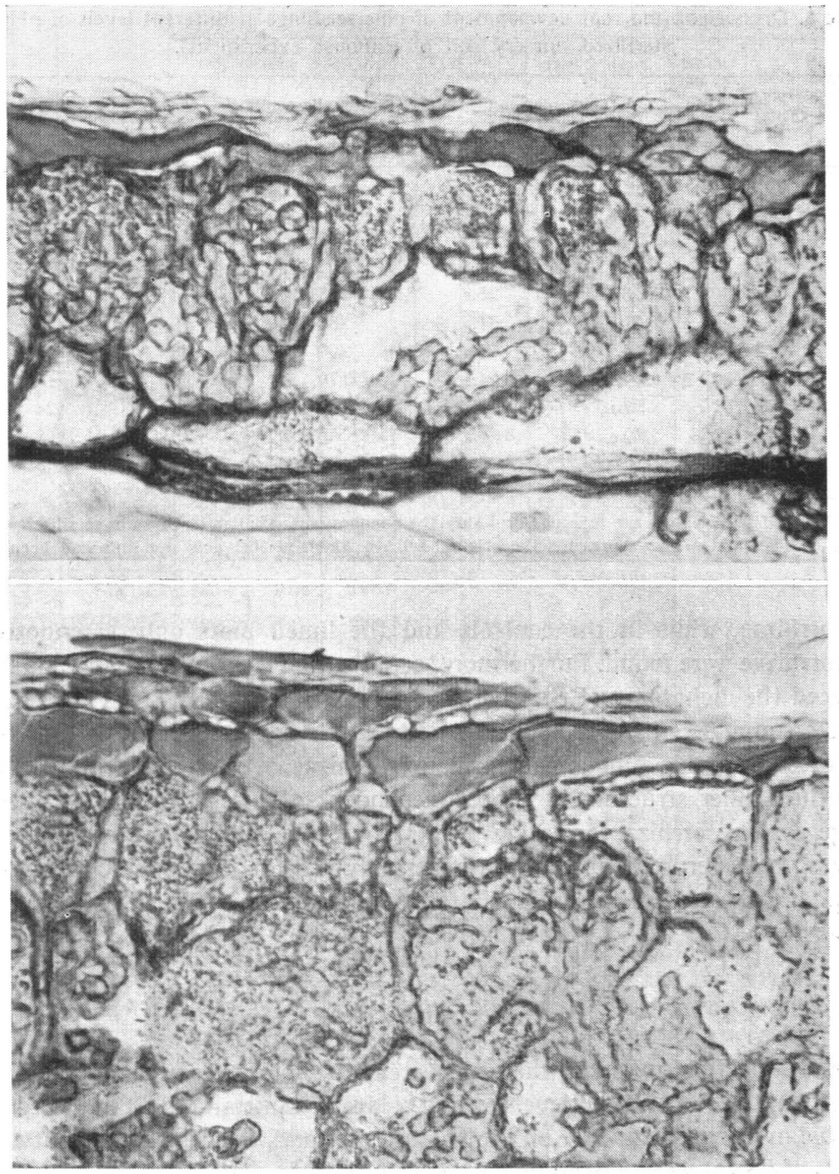


Fig. 13. Ectendotrophic structure of mycorrhizae grown in peat at widely different pH values.

a. initial pH 2.2
b. » 12.6

Table 8. Dry weight and root development of pine seedlings at different levels of pH in unsterilized nursery soil (greenhouse experiment).

Final pH [1]	Dry weight mg		No. of short root tips per seedling	Tips of dichotomous short roots	
	Shoot	Root		No.	%
3.3	25.4	9.7	90	7	8
3.9	36.5	22.4	297	114	38
4.2	77.4	37.8	302	98	32
4.6	58.5	25.6	245	114	47
5.2	61.8	31.9	288	79	27
7.2	44.7	20.2	200	48	24
7.5	44.7	21.3	170	30	18
7.7	37.1	18.1	139	34	24
8.0	20.0	9.1	59	5	8
11.4	20.7	9.8	65	2	3

[1] The initial pH values are missing. Probably the changes were similar to those in sterilized soil (see Table 7). The final pH 5.2 represents the original soil, to which neither acid nor lime had been added.

mycorrhizae, while in the controls and the limed plots only ectendotrophic mycorrhizae were found. Furthermore, acidification clearly promoted and liming reduced the dichotomy of the short roots.

As a summary of the ecological experiments a conclusion can be drawn that the ectendotrophic fungus can form mycorrhizae over a very wide pH range and their inner structure is fairly independent of the soil pH. The optimum range for mycorrhiza formation is pH 4—6. Soil alkalinity, as created by liming, however, probably still further inhibits the ectotrophic mycorrhizal fungi and therefore a high soil pH favors the predominance of ectendotrophic mycorrhizae.

Fertilization

In Hyytiälä old nursery, where the ectendotrophic mycorrhiza first attracted attention, the soil nutrient level was fairly low. BJÖRKMAN (1942) found ectendotrophic mycorrhizae mainly on the poorest sites and, likewise, in the pot experiments of BERGEMANN (1955) mycorrhizae with intracellular hyphae and without a mantle appeared in a poor sandy soil. On the other hand, the nursery survey (pp. 23—25) did not reveal any correlation between soil fertility and the presence of the ectendotrophic mycorrhiza.

In the following fertilization experiment the same nursery soil and peat were used as in the other pot experiments of 1963 and, in addition, quartz sand. Strain E—57 was used for inoculation, and the nutrients were given in the form of ammonium sulfate, double superphosphate, and potassium sulfate;

Table 9. Dry weight and root development of pine seedlings grown in sterilized soil under different fertilizer treatments with and without fungal inoculation.

Soil	Treatment	Control					Inoculated				
		Dry weight mg		No. of short root tips	Tips of dichotomous short roots		Dry weight mg		No. of short root tips	Tips of dichotomous short roots	
		Shoot	Root		No.	%	Shoot	Root		No.	%
Nursery soil	O	35.6	24.6	251	2	1	35.6	26.5	253	78	31
	N_1	53.5	35.6	272	16	6	104.4	66.0	391	42	11
	N_2						66.6	32.1	195	23	12
	N_5	62.3	29.0	243	1	△	67.8	28.8	204	44	22
	N_{10}						47.0	22.1	184	8	4
	P_1	42.8	47.4	556	64	12	47.9	48.6	385	90	23
	P_2	60.4	49.9	530	73	14	46.4	41.4	327	94	29
	P_5	53.8	35.1	321	51	16	42.7	38.3	307	62	20
	P_{10}	35.8	41.2	301	32	11	32.5	25.3	267	98	37
	K_1	51.0	50.9	371	51	14	45.3	36.5	241	46	19
	K_2	65.0	47.0	388	65	17	46.2	31.8	254	67	26
	K_5	49.2	48.5	443	40	9	36.6	26.9	242	73	30
	K_{10}	28.0	21.2	198	20	10	35.7	24.9	221	54	24
	NP_1	89.6	53.4	247	35	14	125.4	75.6	372	75	20
	NP_2	83.2	54.4	284	27	10	106.5	67.2	286	4	1
	NP_5						88.0	31.8	176	1	△
	NPK_1	83.4	58.3	269	21	8	74.6	53.4	265	12	5
	NPK_2	54.6	29.7	141	—	—	39.8	21.5	92	3	3
	NPK_5						53.8	24.1	152	7	5
Peat	O	35.7	28.5	325	9	3	36.4	27.2	255	45	18
	N_2	69.8	47.7	469	19	4	82.7	47.4	382	63	16
	N_5	65.0	36.5	198	13	7	53.5	29.1	150	16	11
	N_{10}						53.2	23.6	140	22	16
	P_2	29.8	24.3	321	14	4	38.0	30.6	329	91	28
	P_5	39.0	35.7	393	7	2	38.1	48.1	407	126	31
	P_{10}	33.6	29.0	270	14	5	32.1	24.0	293	48	16
	P_{20}	25.0	22.6	300	14	5	32.0	27.4	231	15	6
	K_2	33.4	28.7	414	40	10	35.6	28.0	394	90	23
	K_5	33.4	23.8	372	31	8	31.2	24.0	378	103	27
	K_{10}	25.6	19.6	179	17	9	30.1	24.8	225	73	32
	K_{20}	30.6	24.6	394	15	4	29.6	18.8	262	61	23
	NP_2	57.6	33.5	120	1	1	61.4	37.7	285	88	31
	NP_5	59.6	32.0	297	20	7	56.4	28.6	177	14	8
	NP_{10}	44.0	30.8	168	2	1	43.7	22.4	146	8	5
	NP_{20}						54.6	25.8	188	1	1
	NPK_2						47.7	32.6	150	11	7
	NPK_5	61.5	33.5	112	4	4	48.3	30.2	347	51	15
	NPK_{10}	50.0	22.1	122	4	3	44.6	23.7	125	10	8
Quartz sand	N_{05}	36.8	18.1	169	1	—	29.4	19.6	196	83	42
	N_2						32.2	19.8	178	88	49
	P_{05}	21.6	17.1	181	4	2	25.6	16.4	170	18	11
	P_2	21.6	14.7	110	—	—	24.2	14.1	130	15	12
	NPK_{05}	21.5	10.0	89	24	27	21.4	11.7	117	44	38
	NPK_1						36.8	25.9	271	133	49

the amounts are calculated in tons of fertilizer per hectare, 0.5 g per pot corresponding to 1 ton per hectare. Thus, for instance, N_{05} (cf. Table 9) means 0.5 ton of ammonium sulfate per hectare, and NPK_5 means 5 tons of ammonium sulfate, 5 tons of superphosphate, and 5 tons of potassium sulfate per hectare. The fertilizers were added to the pots in solution immediately after seeding, with the exception of the quartz sand pots, where the fertilizers were added later in two or three doses.

The size of the seedlings and their short root relations at harvesting (Oct. 6) are presented in Table 9. There was always one control pot (5 seedlings) and 2 inoculated pots (10 seedlings) per treatment.

As in fertilizing experiments in general, so here heavy nitrogen fertilization increased shoot growth and decreased root growth. Likewise the number of short roots and their dichotomous branching were reduced with increasing nitrogen fertilization. Phosphorus and potassium promoted the formation of short roots and their dichotomy even in uninoculated seedlings.

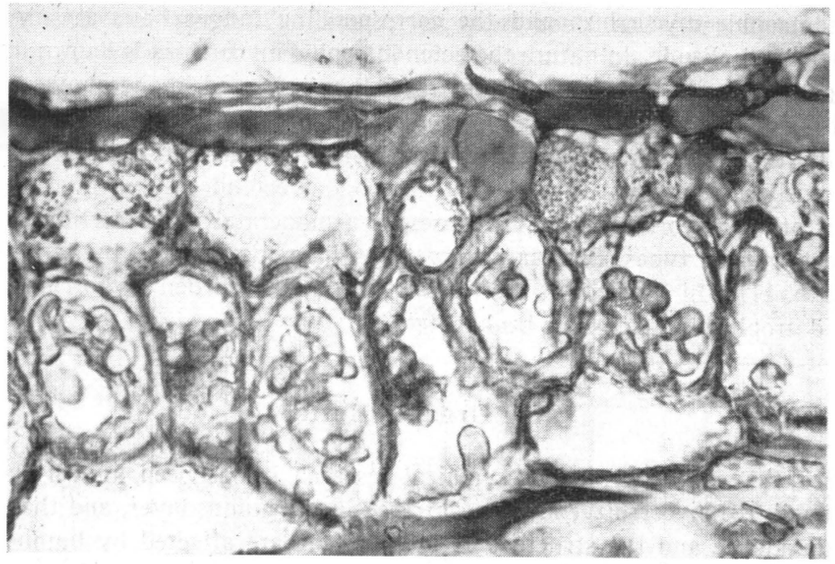
Mycorrhizal relations. Again, in all the inoculated pots, independently of fertilizer treatments, inoculation resulted in mycorrhizal infection. The structure of mycorrhizae was very much the same in unfertilized and in differently fertilized pots, and likewise the differences of mycorrhizal structure in different soils (nursery soil, quartz sand, and peat) were small (Fig. 14). It was noticed, however, that in the pots which had received heavy nitrogen fertilization (N_5, N_{10}, NP_{10}, NP_{20}, NPK_5, and NPK_{10}), the mycorrhizae had fewer intracellular mycelia and the intracellular infection was concentrated mainly in the basal part of the short roots, while in the apical part only a coarse Hartig net and a thin mantle were present.

Greenhouse experiments, which were conducted in 1962 and 1963 in unsterilized soil, led to similar conclusions. Heavy nitrogen fertilization reduced mycorrhizal development — as judged from the dichotomy of short roots or microscopically — but even the strongest application (10 g ammonium sulfate per pot, corresponding to 30 tons per hectare), which was definitely harmful to the seedlings, could not completely prevent the formation of mycorrhizae. At heavy nitrogen fertilization the mycorrhizae also had relatively less intracellular mycelia, as was the case in sterilized soil, too.

Similar conclusions were also made in a nursery experiment when the development of the seedlings was observed in two successive summers. Strong nitrogen application (5—20 tons of ammonium sulfate per hectare) reduced both the dichotomy of the short roots and the mycorrhizal infection but could not prevent the formation of ectendotrophic mycorrhizae. Further, an observation was made that moderate nitrogen fertilization (500—2 000 kg per hectare) promoted the ectotrophic mycorrhiza over the ectendotrophic one in the second summer.

Thus, the fertilization experiments showed that regarding soil fertility, the

a



b

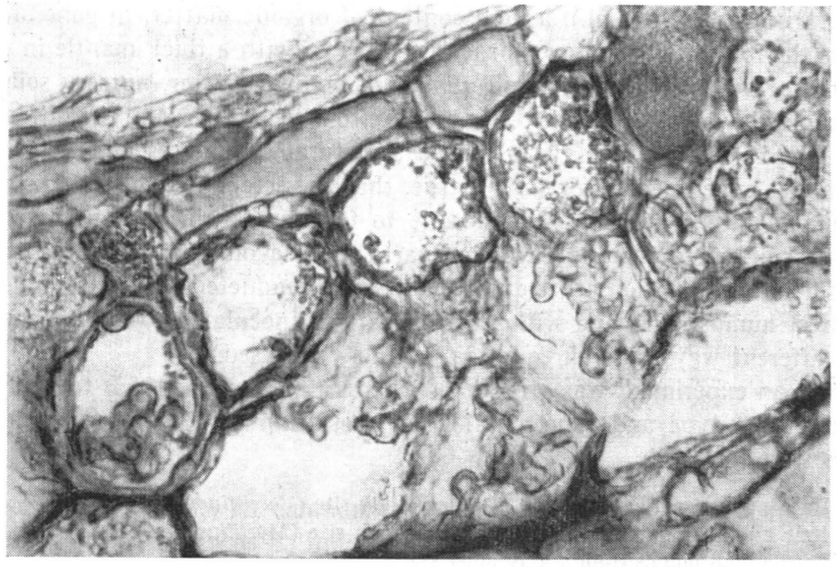


Fig. 14. Ectendotrophic structures of mycorrhizae grown in different soils and at different levels of fertility.

a. Quartz sand, N_{05}
b. Peat, NPK_{10}

ectendotrophic mycorrhiza and the corresponding fungus have a very wide ecological amplitude. In nature the ectendotrophic mycorrhiza is known mainly on poor sites, and in very poor substrates it was formed in the above experiments, but morphologically almost identical mycorrhizae also occurred in heavily fertilized pots and nursery beds. Strong nitrogen fertilization, however, apparently reduces the intracellular infection of ectendotrophic mycorrhizae, and if other mycorrhizal fungi are present a moderately high fertility favors the ectotrophic types. Similar observations have been made by BJÖRKMAN (1942, p. 111: »In Töpfen mit kräftigem Nitratzusatz wurden Mykorrhizen von ektendotrophem Typ niemals beobachtet.»).

Soil Organic Matter

Since the early days of mycorrhizal research it has been known that in forest soil tree mycorrhizae are concentrated in the humus layer, and that both the abundance and the structure of mycorrhizae are affected by humus properties (MELIN 1927; BJÖRKMAN 1942, 1949, 1956; HARLEY 1948; DOMINIK 1963; MIKOLA 1963; et al.); a high content of organic matter, in general, promotes the formation of mycorrhizae and of those with a thick mantle in particular. Regarding the content and quality of organic matter, nursery soils and the humus layer of forest soils differ greatly from each other, and the differences might be correlated with the observed structural differences of mycorrhizae. The above experiment showed, it is true, that the ectendotrophic fungus, when inoculated into sterilized soil, was able to form mycorrhizae equally well in nursery soil and in peat, and even in quartz sand devoid of any organic matter. For further information other experiments were conducted with different types of forest humus, with and without sterilization. Inoculation was performed in two different ways, and three fungal strains were used.

First, an experiment was arranged with sterilized soil (in 1963) to study the effect of the type and concentration of forest humus. The following humus types were included:

a) Raw humus from a pine stand of a dry sandy site (*Vaccinium* type)
b) Raw humus from a spruce stand of a medium site (*Myrtillus* type).
c) Fertile mull humus from a gray alder stand
d) Milled peat (the same as in all the other experiments).

The humuses were mixed with sand in the following proportions (by volume): 100:0, 75:25, 50:50, 25:75, and 0:100. The strain E—57 served as inoculum; two inoculated pots and one control per treatment were included.

The development of the seedlings in response to the different treatments is presented in Table 10. It will be noticed, that the seedlings grew in fertile mull much better than in the other humus types, while differenced between the

other types were small. The number of short roots and their dichotomous branching were greatest in the mull, where, in addition, dichotomy of short roots was relatively common even without inoculation or outside contamination.

Table 10. Dry weight and root development of pine seedlings grown in different types and concentrations of sterilized humus without and with fungal inoculation.

Type of humus	Content of humus %	Control					Inoculated				
		Dry weight mg		No. of short root tips	Tips of dichotomous shoot roots		Dry weight mg		No. of short root tips	Tips of dichotomous short roots	
		Shoot	Root		No.	%	Shoot	Root		No.	%
Mull	100	58.5	47.4	334	49	15	62.6	46.6	556	178	32
	75	45.8	46.5	403	31	8	57.0	36.4	276	114	41
	50	39.8	37.8	245	10	4	46.4	33.8	287	94	33
	25	28.6	26.6	287	39	14	37.3	28.9	249	63	25
	0	23.7	15.1	152	1	△	26.7	16.2	150	17	11
Spruce raw humus	100	29.1	22.1	220	21	10	31.9	26.9	280	86	31
	75	24.0	16.6	83	2	2	27.0	22.7	193	35	18
	50	22.6	19.7	175	4	2	29.0	18.8	178	40	22
	25	25.2	22.4	196	—	—	30.3	17.2	144	29	20
	0	23.7	15.1	152	1	△	26.7	16.2	150	17	11
Pine raw humus	100	24.3	18.0	297	14	5	2.25	15.1	268	44	16
	75	24.2	21.2	154	1	△	23.0	16.8	270	51	19
	50	20.6	16.7	152	—	—	29.5	11.7	140	18	13
	25	28.6	21.2	177	—	—	29.2	16.8	264	29	11
	0	23.7	15.1	152	1	△	26.7	16.2	150	17	11
Peat	100	31.5	31.2	262	29	11	34.4	28.9	253	36	14
	75	25.4	19.0	302	—	—	28.6	22.1	347	41	12
	50	30.4	23.4	262	1	△	27.5	19.0	232	45	19
	25	37.2	23.8	299	—	—	27.0	23.2	285	32	11
	0	23.7	15.1	152	1	△	26.7	16.2	150	17	11

Mycorrhizal relations. The abundance of dichotomous short roots on inoculated seedlings is often taken as an indication of the degree of mycorrhizal development. In all the inoculated pots dichotomous short roots and ectendotrophic infection were already present at the first sampling (Aug. 27). Likewise, the structure of the mycorrhizae was very similar in all the treatments. It was only in pots with a high humus content that the mycorrhizae usually had a thin mantle and were, on the average, somewhat thicker (35—40 μ) than in sand (30—35 μ) where the mantle was missing.

In the mixtures of peat and sand, inoculation was also performed with two other strains, E—15 and E—35, and with the latter of these two methods

were tested, viz. besides the ordinary pure culture inoculation, soil from a pot of a previous semiaseptic inoculation experiment (p. 17) was used as inoculum (series E—35p, Table 11). There were no differences in the size or number of short roots between seedlings inoculated with the different fungal strains. Therefore, only results regarding the dichotomous short roots are presented (Table 11). Even here it will be noticed that dichotomy is more profuse in peat and in mixtures of peat and sand than in plain sand. The dichotomy and the structure of the mycorrhizae were the same, irrespective of the fungal strain used. When soil had been used for inoculation (E—35 p.), infection had probably taken place somewhat slower and, therefore, the seedlings in the pots marked E—35p had fewer dichotomous mycorrhizae than the seedlings inoculated with pure cultures.

Table 11. Dichotomous short roots in pine seedlings grown in mixtures of peat and sand when inoculated with different fungal strains.

Peat	Sand	Control		Inoculated							
				E—15		E—35		E—35 p		E—57	
Vol. %		No.	%	No.	%	No.	%	No.	%	No.	%
100	0	29	11	80	26	92	28	39	10	36	14
75	25	—	—	54	15	34	12	17	5	41	12
50	50	1	△	53	16	40	17	26	7	45	19
25	75	—	—	62	21	74	28	28	12	32	11
0	100	1	△	11	6	28	12	16	10	17	11

The above soils were also used in an experiment without sterilization and inoculation. Otherwise the experimental conditions were the same. The short root relations of the seedlings at the end of the growing season are presented in Table 12. Regarding the structure of short roots, in sand there were no mycorrhizae present, but the short roots were pseudomycorrhizal, i.e. infected by thin intracellular hyphae. In quartz sand the short roots were non-mycorrhizal and uninfected. In the natural forest soils the short roots were ectotrophic mycorrhizae with a mantle; occasionally some intracellular infection was also present, which, however, was probably caused by some other fungus than the one with which this paper is concerned. In mull all the mycorrhizae were light-colored while in spruce raw humus there were numerous black *Cenococcum* mycorrhizae and in pine raw humus a few. In peat the mycorrhizal infection was probably air-borne; the predominant type there was an ectotrophic mycorrhiza with a thin mantle, with clamp connections on the hyphae.

The influence of soil organic matter on mycorrhizae in unsterilized soil was further studied by mixing the soil of Hyytiälä old nursery with spruce raw humus and with peat. Pots of 650 cm³ capacity were used in this experiment,

Table 12. Short root development in different soils without sterilization and inoculation.

Soil	Number of short root tips	Tips of dichotomous short roots	
		No.	%
Mull	440	179	41
Spruce raw humus	190	32	17
Pine raw humus	283	63	22
Sand	121	—	—
Peat	258	94	36
Nursery soil	205	89	43
Quartz sand	64	2	3

and each treatment was represented by one pot (5 seedlings). The development of the seedlings is shown in Table 13. In many respects the results differ from those of some other experiments. Thus, the spruce raw humus was a fairly favorable substrate, while in smaller pots and after sterilization it resulted in poor growth (cf. Table 10). It is also surprising that both the actual and the relative number of dichotomous short roots decreased along with increasing humus content.

Table 13. Dry weight and root development of pine seedlings grown in different mixtures of nursery soil and humus.

Percentage of humus	Nursery soil + Spruce raw humus					Nursery soil + Peat				
	Dry weight mg		No. of short root tips	Tips of dichotomous short roots		Dry weight mg		No. of short root tips	Tips of dichotomous short roots	
	Shoot	Root		No.	%	Shoot	Root		No.	%
10	32.5	19.8	213	105	49	28.3	17.7	229	101	44
30	49.6	25.1	209	102	49	36.0	34.8	262	163	62
50	56.0	31.9	215	88	41	30.6	20.7	262	175	67
70	61.7	36.3	226	71	31	29.0	23.9	312	129	41
90	64.6	41.8	233	22	9	24.0	18.7	262	137	52
100	84.5	43.6	230	11	5	26.0	17.7	259	71	27

The structure of the mycorrhizae was interesting. In all the pots with mixtures of nursery soil and peat, irrespective of their proportions, only typical ectendotrophic mycorrhizae were present. In pure peat, where the mycorrhizal infection was apparently air-borne, two kinds of mycorrhizae were found, viz. typical ectendotrophic mycorrhizae and ectotrophic ones with a thick mantle. In addition, on one ectendotrophic mycorrhiza a secondary mantle of *Cenococcum* was noticed. In the mixture of nursery soil and spruce raw humus all the my-

corrhizae were ectendotrophic if the content of forest humus was 50 % or less. In the pots containing 70—90 % forest humus, both ectotrophic and ectendotrophic mycorrhizae were present. Ectotrophic mycorrhizae were formed by several fungal species; thus, in the mantles black *Cenococcum* hyphae and light-colored hyphae both with and without clamp connections were distinguishable, and even coarse dark hyphae with clamp connections. In pure forest humus ectendotrophic mycorrhizae were missing, i.e. all the mycorrhizae were ectotrophic, having a mantle.

Accordingly, the ectendotrophic fungus is able to infect young pine seedlings rapidly even in the presence of other mycorrhizal fungi.

Effect of Inoculation

The presence of dichotomous short roots in pine is usually considered an indication of mycorrhizal association, and their abundance has been used to express the degree of mycotrophy (e.g. RICHARDS & WILSON 1963; LAIHO & MIKOLA 1964); the same usage was partly adopted above. As was seen, however, dichotomous branching was also relatively common on uninoculated seedlings in sterilized soil, where not even thorough microscopic examination could reveal any fungal infection.

The literature contains numerous references to dichotomous branching of non-mycorrhizal short roots. In pure cultures it was first discovered by MELIN (1925) and HATCH (1937), and SLANKIS (1948, 1951, etc.) has studied the physiological factors affecting the dichotomous branching under aseptic conditions. Pseudomycorrhizae of pine, i.e. short roots infested by non-mycorrhizal fungi, are often dichotomous in nature, although not to such a degree as true mycorrhizae (MELIN 1923a, 1927; BJÖRKMAN 1942). Uninfected, non-mycorrhizal short roots in natural soils were described by HATCH & DOAK (1933), WERLICH & LYR (1957), and Goss (1960). Goss remarks that in pine »dichotomy is an inherent morphological characteristic which becomes more pronounced by fungus association». As SLANKIS had shown with pure cultures, so LEVISOHN (1952, 1960) demonstrated under non-aseptic conditions that root dichotomy is promoted by exudates of mycorrhizal fungi and by specific growth substances. Likewise LEVISOHN (1954, 1960) pointed out that the dichotomy is also strongly increased by the »haustorial fungus infection», which she calls pseudomycorrhizal but is probably identical with the ectendotrophic association of the present paper. It is not possible here to discuss in detail all the factors which may cause or favor the dichotomous branching of uninfected short roots in sterilized soil. Addition of organic matter, for instance, had such an effect (Table 10). It is noteworthy, however, that some dichotomy occurred even in quartz sand devoid of any organic matter. A distinct positive influence on root forking was exerted by phosphorus and potassium fertilization (Table 9). Different kinds of

forest humus also had different effects, that of fertile mull being the strongest (Table 10). Incidentally, for some unknown reason forking was sometimes exceptionally intense (e.g. Table 7, »nursery soil, pH 5.3»). The stimulating effect of fungal inoculation, however, was easily distinguishable in all the experiments with sterilized soil (Tables 6—7 and 9—11).

The ectendotrophic mycorrhiza has often been found and described on slow-growing or stunted seedlings and, therefore, the view has been expressed that it represents a special type of ectotrophic mycorrhiza, where the symbiotic balance is disturbed and the fungus behaves as a more or less harmful parasite. Experimental evidence to support such an assumption, however, is insufficient or open to criticism. Neither can the above experiments, when pine seedlings were grown in sterilized soil and ectendotrophic mycorrhizal association was established by pure culture inoculation, answer the question of whether the fungal infection is beneficial or harmful to the seedlings, for in most cases (Tables 6—7 and 9—11) there was no significant difference in the size of the mycorrhizal and non-mycorrhizal seedlings. Neither did these experiments provide any opportunity to compare the ectendotrophic fungus with ordinary ectotrophic fungi in regard to their influence on the growth of the host plant.

Considering the experimental conditions, a distinct effect of the fungal infection on the host could hardly be expected. The whole duration was one growth season only, inoculation was done at the end of July and fungal infection probably took place by the end of August. Thus, the mycorrhizal state lasted for little more than a month and occurred at a time when the main growth period was over. Several former studies (e.g. Goss 1960; LAIHO & MIKOLA 1964) have also shown that mycorrhizal infection is of little importance in the development of seedlings in their first season, because infection takes place in the late summer when the shoots have largely completed their annual growth. Deficiency symptoms due to lack of mycorrhizae have been reported to appear in pine simultaneously with the first fascicle needles (MCCOMB 1943), i.e. in Scotch pine in the second summer. Likewise, LEVISOHN (1954) did not notice any harmful effect of the »haustorial» infection until the second summer.

There are a few cases, however, in which the ectendotrophic mycorrhiza seemingly exerted a favorable effect on seedling growth. Such an indication was observed in the experiment when seedlings were grown in mixtures of sand and forest humus (Table 10, Fig. 15). Analysis of variance showed that the difference in weight between inoculated and uninoculated seedlings was statistically significant in the mixtures of sand and mull ($F = 5.6^*$) and in the mixtures of sand and spruce raw humus ($F = 7.4^*$).

Although the above instances do not conclusively prove the beneficial effect of the ectendotrophic infection, there is at least no indication to suggest a harmful effect either. More striking results indicating the benefit of ectendotrophic mycorrhizae were obtained by LAIHO (1965).

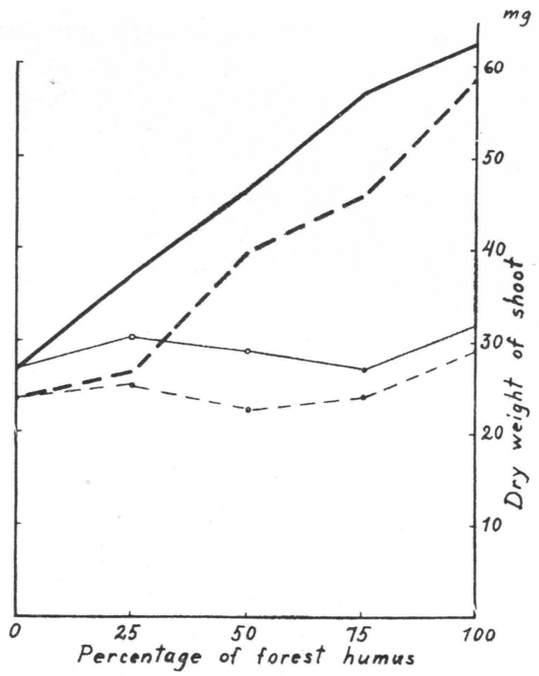


Fig. 15. Shoot dry weight of pine seedlings grown in the mixture of sand and forest humus.
Block lines: Sand + mull
Thin lines: Sand + spruce raw humus
Solid lines: Inoculated with E—57
Broken lines: Without inoculation

Discussion

The ectendotrophic mycorrhiza as described in this paper has proved very common on pine in Finnish nurseries. The same type of mycorrhiza, probably formed by the same fungus, is also common in forest nurseries elsewhere (LAIHO 1965). In all probability this is the type of infection which LEVISOHN (1954, 1963) found to be common in many English nurseries and which was called by her the »haustorial type of intracellular root infection» or pseudomycorrhiza.

The ectendotrophic mycorrhiza of this paper and the haustorial infection of LEVISOHN have the following features in common:

1. As fas as can be concluded from published microphotographs, the anatomy of the fungus-root association is quite similar, both in the mycorrhizae and in the cortex of long roots.
2. The macroscopic appearance of the mycorrhizae is the same. LEVISOHN, it is true, refers to the complete absence of a fungal mantle, while the mycorrhizae of this study often had a thin mantle. The presence or absence of a mantle, however, probably depends on environmental conditions.
3. The anatomy of the hyphae and the appearance of pure culture colonies show a great similarity. (According to LEVISOHN the colonies on agar are »dark brown», while the colonies in this study could rather be described as »light brown»; the difference may be due to different substrates. In another connection LEVISOHN describes the hyphae as »brownish».)
4. The typical ectendotrophic or »haustorial» infection occurs on pine only, while the same fungus on spruce forms mycorrhizae with a coarse intercellular net but without a mantle or intracellular hyphae.
5. Both the ectendotrophic and the »haustorial» infection are characteristic of forest nurseries on soils of agricultural type, while they are rare or lacking in natural forest soils.

In all probability the ectendotrophic mycorrhizae of BJÖRKMAN (1940, 1942) and Goss (1960) were formed by the same fungus.

According to LEVISOHN (1954), the »haustorial» fungus greatly resembles the root fungus *Rhizoctonia silvestris*, described by MELIN (1923a), and probably belongs to the same genus. The ectendotrophic fungus and MELIN's *Rhizoctonia* are very different in many respects, however, although morphologically the hyphae appear rather similar. Thus, MELIN isolated *Rhizoctonia* from black or dark mycorrhizae, while the ectendotrophic mycorrhizae of the present study are as light-colored as uninfected short roots. Likewise the colony on agar is light brown while the colony of *Rhizoctonia silvestris*, according to MELIN's description, is gray or almost black. Sclerotia or sclerotia-like formations were never observed, either on the surface of mycorrhizae or on agar. Furthermore, in physiological pure culture experiments the isolated organism behaved like mycorrhizal fungi and unlike *Rhizoctonia silvestris*. Thus, for instance, the ectendotrophic fungus had a relatively narrow pH range, while *Rhizoctonia silvestris* is more indifferent to pH (MELIN 1925, p. 17). So far the ectendotrophic fungus is unnamed and its position in the fungal system is unknown, an affinity with *Rhizoctonia*, however, being unlikely.

Several authors suggest that the ectendotrophic mycorrhiza is a formation in which the symbiotic balance of ectotrophic association has been disturbed, the fungus is more or less parasitic, and thus the association may harm rather than benefit the host plant. Such an idea is based on the common observation that ectendotrophic mycorrhizae are often found on stunted and slow-growing seedlings. According to this hypothesis, the same fungal species would be able to form both ectotrophic and ectendotrophic mycorrhizae, depending on environmental conditions. Regarding the ectendotrophic fungus of this study the above hypothesis does not hold, for the inner structure of the mycorrhizae was essentially the same under widely varied environmental conditions. On the other

hand, the type of infection was closely dependent on the host species: in pine the infection is both inter- and intracellular, but in spruce intercellular only; on both tree species the mantle is thin or lacking.

The possible existence of fungi which can form both ectotrophic and ectendotrophic mycorrhizae even on the same tree species, is not, of course, excluded. On the contrary, the literature includes several references to intracellular infection caused by some fungus other than the ectendotrophic one of this study. Thus, in the ectendotrophic mycorrhizae which have been described on spruce (e.g. BERGEMANN 1955) the fungal partner must be another species, as well as in the birch mycorrhizae described by MELIN (1923b); in pine ectendotrophic mycorrhizae with a thick mantle have even been reported (MELIN 1923a), and intracellular hyphae can also be found in the black mycorrhizae formed by the well-known mycorrhizal fungus *Cenococcum graniforme*. The commonest ectendotrophic mycorrhiza, however, is the particular type which is dominant in many nurseries and is the object of the present study; in all probability this fungus never forms ectotrophic mycorrhizae in pine.

There still remains the question of whether perhaps the ectendotrophic fungus is more parasitic and less beneficial for the host tree than the ectotrophic ones, as has been reported by LEVISOHN (1954). According to her, the »haustorial» fungus is a one-sided parasite and consequently the association is not symbiotic; therefore, such root structures cannot be called mycorrhizae but pseudomycorrhizae — provided the use of the latter term is accepted (LEVISOHN 1963). She also published some experimental evidence in support of this opinion. In a later paper (LEVISOHN & PARRY 1960), however, she has admitted that heavy intracellular infection also occurs on healthy and thriving seedlings when the association is symbiotic. Then the fungal associate, however, is suggested to be a different species.

In the present study nothing emerged to suggest that the ectendotrophic fungus was more parasitic than the other mycorrhizal fungi. It is true, digestion of hyphae in the cortex cells could not be proved with certainty, but a symbiotic relationship with an intracellular fungus even is possible without digestion of hyphae. The nursery survey (pp. 23—25) showed that no correlation existed between the size and vigor of the seedlings and the presence of ectendotrophic infection, and in greenhouse experiments uninoculated seedlings and seedlings inoculated with the ectendotrophic fungus grew equally well, any differences being in favor of the inoculated seedlings (pp. 49—50). The question of whether the ectendotrophic fungus is more parasitic and less beneficial than the ectotrophic mycorrhizal fungi should be studied with long-term field experiments under conditions in which the soil initially contains no mycorrhizal fungi.

Two conspicuous features are characteristic of the ectendotrophic pine mycorrhiza, viz. it is almost exclusively confined (1) to young (1—3-year-old) seedlings, and (2) to nursery soils. Possible reasons for such an occurrence have been discussed above, but without any definite conclusion. The possibility was suggested that the fungus might not be so dependent on the light received by the host as the ectotrophic fungi, and could therefore rapidly infect even young seedlings, the photosynthetic capacity of which is still relatively low. Whether this is so or not, there still remains the question of why the ectendotrophic fungus later on disappears and is replaced by ectotrophic fungi.

As the above experiments (pp. 32—48) indicate, the ectendotrophic mycorrhizal fungus has a very wide ecological amplitude in regard to light intensity and soil fertility, acidity, and humus content. LEVISOHN (1954), in addition, observed that the respective mycorrhiza prevails in water-logged soil, i.e. under semianaerobic conditions. In spite of the wide ecological amplitude, however, the ectendotrophic fungus has a weak competitive ability in natural forest soils against the indigenous fungal population. So far, however, the factors which in forest soils favor the indigenous fungi and inhibit the ectendotrophic mycorrhiza former are unknown.

As was shown before, when seedlings are transplanted from the nursery into forest soil their mycorrhizal population is largely changed. Consequently the question arises of whether or how this phenomenon may have to be considered in forestry practice. Several authors have pointed out that such a mycorrhizal association should be established in the nursery, which would be best suited for the future field conditions (BJÖRKMAN 1944, 1956; MOSER 1956). If the ectendotrophic fungus is present in the nursery, however, it seems invariably to infect pine seedlings, which consequently have ectendotrophic mycorrhizae at the time of transplanting. Inoculation of seedlings with »forest fungi» in a nursery, where the ectendotrophic fungus is present, hardly is feasible. The change of the mycorrhizal associate after transplanting, however, seems to take place easily in pine, at least in natural forest soil containing indigenous mycorrhizal fungi. If seedlings are transplanted into agricultural or other non-forest soil, the growth of the ectendotrophic fungus may continue in the new environment, as has probably happened on some grassland plantations (Goss 1960). Likewise spruce mycorrhizae, although ectotrophic, generally might be formed in nurseries by the same ectendotrophic fungus. The question of whether in spruce the root associate is also easily changed after transplanting or whether there are perhaps disturbances, which might account for stagnation of the transplants, remains outside the scope of this study.

The great ease with which the ectendotrophic fungus can be inoculated into seedlings in sterilized soil renders it a suitable test organism for different kinds of mycorrhizal investigations.

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